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SYMPATHETIC VASODILATOR FIBRES

J. H. BURN

Pharmacological Laboratory, College of the Pharmaceutical Society, London

Central and local control. In the course of the Dohme lectures for 1933, Dale contrasted the vasoconstrictor mechanisms of the body with the vasodilator mechanisms, in that the former are for the most part centrally controlled, while the latter are locally controlled. In the introduction to Gaddum's book, *Gefässerweiternde Stoffe der Gewebe*, Dale has emphasised this contrast; he points out that the constrictor regulation is exerted through the vasoconstrictor nerves and also by the liberation of adrenaline or vasopressin, while the dilator regulation is exerted through axon reflexes at the termination of sensory nerves, or by local liberation of vasodilator substances. Apart from centrally controlled dilator nerves to special organs, such as the chorda tympani and the nervi erigentes, Dale concludes that there is no provision for active nervous dilatation to satisfy simultaneously the needs of the whole body. According to this view sympathetic vasodilator fibres would be of little functional importance. We could regard their existence as due to an evolutionary error which, had it gone very far, would have rendered useless the sympathetic vasoconstrictor fibres; fortunately, however, the error did not develop, and the sympathetic vasodilator fibres remained a mechanism having no more value than the human vermiform appendix.

There is, however, another possibility. The view as presented is based in essential points on the physiology of the cat, and it may be unjustifiable to suppose that a system which is simple and logical for that animal is the perfect system for other animals. If our main experimental animal was the mole, a hidebound experimentalist might conclude that the eye was an unimportant sense organ. There is no inherent difficulty in supposing that in larger animals than the cat there is a sympathetic dilator mechanism acting nearer the periphery than the main constrictor fibres; through this dilator mechanism, blood supply to muscles (whether skeletal or visceral) can be increased once the constrictor fibres have fulfilled their

function of providing for the heart and brain. This mechanism may be submerged in the cat, but be of more importance for other animals, e.g., for animals of the chase, like the dog and the hare.

METHODS. Little need be said about the methods used for detecting changes in the blood flow in limbs; none of them are very satisfactory. Some observers have been content to assume vasodilatation because stimulation of the lower abdominal sympathetic caused a fall in the general blood pressure. Many have used the plethysmograph. Plethysmograph records should always show the volume pulse of the enclosed organ, and if they do not, it can be said at once that the plethysmograph is not properly applied; the volume pulse of the leg, or the spleen or the intestine is easily recorded (see Ranson and Wightman, 1922). Even so the plethysmograph record cannot be trusted to show the changes in the blood flow through the organ, unless the record moves from moment to moment in the opposite sense to the general blood pressure. Evidence from the plethysmograph should be supported whenever possible by evidence obtained in other ways, for example by records of venous outflow, or by evidence obtained from perfusion experiments in which the inflow and outflow are measured. Changes in the flow through the skin or the muscles have been observed by the use of a thermocouple either applied to the skin, or inserted into the muscle (Wybauw, 1936). Much work has been done by Rein and his colleagues, and also by others, with the thermostromuhr. It is an instrument of great sensitiveness which gives beautiful records; so much so that some of those who use it appear to think that the thermostromuhr cannot lie, and that observations which conflict with the results it gives can be brushed on one side. A more moderate view seems preferable, for examples will be given later in which the evidence from the thermostromuhr is difficult to interpret.

The use of anesthetics. The use of anesthetics in experiments on the vasomotor system is open to objection. Elliott showed in 1912 that during anesthesia with chloroform, or ether or urethane, there was a discharge of adrenaline from the suprarenal glands. He used cats in which the splanchnic nerves to the suprarenal gland of one side had been cut previously; after some hours' anesthesia, he estimated the amount of adrenaline present in the two glands and found that in the denervated gland there was for example 0.26 mgm., while in the normal gland there was only 0.07 mgm., the rest having been discharged by impulses passing down the splanchnic nerves.

The cardiovascular action of ether was analysed by Cattell in 1923. He showed that in cats the sudden inhalation of a high concentration of

ether vapour often caused a drop in the blood pressure followed by recovery to the previous or to a still higher level. Cattell concluded from experiments with the cardiometer that the drop in blood pressure was due to weakening of the heart and diminished output, while the recovery was due to increased vasoconstriction caused either by a reflex initiated by the fall of pressure or by a direct stimulant action on the vasomotor centre. His evidence for the occurrence of vasoconstriction was obtained from *a*, plethysmograph experiments in which he observed that inhalation of ether caused diminution of limb volume, which was not always attended by fall in blood pressure, and which did not occur if the nerves were severed; *b*, experiments on perfusion rate in which he observed that the time taken for a given volume of saline to flow through a limb, by way of a branch of the main artery during temporary arrest of the blood flow, was increased during ether administration. These results, taken together with those of Elliott, suggest that ether causes a discharge of impulses down all sympathetic nerves. Observations made by Bhatia and Burn (1933) support this suggestion; they examined the effect of ether in cats some hours after decerebration; the suprarenal glands were excluded by ligatures, and records were taken of the spleen volume and of the blood pressure. The cats were artificially respired with air, and when some ether was mixed with the air, a contraction of the spleen volume occurred without change of blood pressure. When the ether was discontinued, the spleen rapidly regained its former volume. Nicotine was then administered until successive doses no longer produced a rise in blood pressure. When the admixture of ether with the respiring air was repeated, there was no longer any effect on the spleen volume, showing that the diminution seen before nicotine was due to sympathetic impulses; there was, however, a large fall of blood pressure, showing that the absence of fall before nicotine was due to vasoconstriction of sympathetic origin which compensated for the weakening of the heart. Thus there is reason to conclude that in animals under anesthetics like chloroform, ether or urethane, the sympathetic system is continuously stimulated by the anesthetic. Herrick, Essex and Baldes (1932), using the thermostromuhr, found that in normal dogs the femoral blood flow was two to three times as great under general anesthesia with ether as it was when the stromuhr was applied under local anesthesia. The rate of flow under general anesthesia was similar to that recorded under local anesthesia after lumbar sympathectomy. These observations suggest that under ether the vasoconstrictor fibres are paralysed, but in view of the previous evidence the possibility must be considered that the greater flow under ether is due to stimulation of

the vasodilator fibres (which, as will be seen, are easily demonstrable in the muscles of the dog) combined with increased cardiac output.

It is true that few physiologists today work with chloroform and ether, but a good many still use urethane, and it is actually fashionable to use derivatives of barbituric acid. Now Brown and McSwiney (1932) have shown that whereas in the spinal cat stimulation of the thoracic sympathetic always leads to inhibition of the musculature of the stomach, after the administration of phenobarbital (Luminal) the effect is reversed and sympathetic stimulation then produces a contraction and rise of internal pressure. Evidently a simple barbiturate is not innocent of other than anesthetic action. Recently Mertens, Rein and Valdecasas (1936) have recorded their inability to observe any effect of adrenaline on the tension of muscle during fatigue; their observations were made on dogs anesthetised with Pernocton (sodium butyl-β-bromallylbarbiturate). Bülbring and Burn (1938) have also investigated the effect of adrenaline on the tension of the muscle of the dog during fatigue, the stimulation being applied to the motor roots; they were able to observe that adrenaline produced a rise in tension in muscles perfused with defibrinated blood, or in the muscles of the spinal dog; they failed, however, to observe any rise of tension in dogs anesthetised with Pernocton. This, therefore, provides a second example of the danger of using derivatives of barbituric acid. Observations made under Dial or Pernocton anesthesia may give a false physiological picture unless confirmed under other conditions.

Early observations on sympathetic vasodilatation. The first observations of Dastre and Morat in 1880 were that stimulation of the cervical sympathetic in the dog caused dilatation of the vessels in the buccal cavity, the mucous membrane of the palate, gums and lips, and in the skin of the lips and cheeks, both in the upper and lower jaws. They recorded that simultaneously the ear and tongue became pale. Similar results were obtained later by Langley and Dickinson in 1890. It is of interest to observe that these fibres supplying the lips and gums, which were the first sympathetic vasodilator fibres to be described, were also the fibres out of which the conception of cholinergic sympathetic fibres grew. In 1931, v. Euler and Gaddum studied the Rogowicz phenomenon, which is the contraction of the lips of the dog produced by stimulating the cervical sympathetic chain after the motor nerve supply of the lips has degenerated. v. Euler and Gaddum found that the fibres which produced flushing of the lips were identical in course and cell station with those which produced contraction of the

lips, and that the contraction was probably due to the liberation of acetylcholine at the endings of these sympathetic vasodilator nerves. This paper had most important consequences.

In 1906 Dale published his classical account of the action of ergotoxine in which he presented evidence for the view that part of its action was to be explained by a paralytic effect on motor sympathetic endings. He showed that sympathetic effects which were purely inhibitory were untouched by the action of ergotoxine, whereas motor effects were either abolished or reversed. When the effect was the abolition of a motor response, he concluded that the innervation was purely motor, but when the effect was reversal of a motor response, he concluded that the sympathetic supply was a mixture of motor and inhibitor fibres, and that the reversal was due to paralysis of the motor component. Those who re-read this paper at the present time cannot help being impressed by the completeness of the evidence advanced by Dale in support of his explanation. After the administration of ergotoxine, either the injection of adrenaline or the stimulation of the splanchnic nerve caused a fall in blood pressure.

Dale, therefore, supposed that there must be vasodilator fibres normally masked by vasoconstrictor fibres. Since the depressor action of splanchnic stimulation might have been due to liberation of adrenaline from the suprarenal glands, he showed in a later paper (1913) that splanchnic stimulation after full doses of ergotoxine was still depressor after removal of the suprarenals, though the effect was then very slight.

Vasodilator action of adrenaline. The depressor action of small doses of adrenaline was first described in the dog by Moore and Purinton in 1900. Dale's observation of the adrenaline reversal produced by ergotoxine suggested that the two depressor effects might be identical in character and that the action of small doses in the absence of ergotoxine might indicate the distribution of the vasodilator nerve supply. Hartman (1915) found that, in cats under urethane, depressor effects were not observed if both subclavian arteries, both carotids and the abdominal aorta above the bifurcation were clamped, but that if these arteries were released and the splanchnic supply was clamped, the depressor effects were greater than in the intact circulation. He concluded that a discharge of adrenaline would give constriction in the splanchnic circulation and dilatation in the peripheral circulation. Hoskins, Gunning and Berry (1916) showed that this peripheral dilatation took place in the muscles rather than in the skin. Gruber (1918) showed that the dilator action was independent of the central nervous system

since it was readily observed in a limb some days after section of the nerves.

The similarity of the depressor effect of small doses of adrenaline to that of larger doses given after ergotoxine is supported by the observations of Rosenblueth and Bradford Cannon (1933) who administered ergotoxine to cats (4 mgm. per kgm.) and observed the effect on the blood pressure of adrenaline before and after clamping the aorta at the diaphragm to exclude the splanchnic area. Before exclusion of the splanchnic area, the effect of adrenaline was pressor; after exclusion it was depressor. Here again the evidence indicated that dilatation occurred more readily somewhere in the system of muscles and skin than in the intestines, as was found by Hartman.

Wyman and Tum Suden (1932) have studied the action of small doses of adrenaline in the rat under urethane and have made the curious observation that they are depressor only if the suprarenal medulla is absent. After the administration of cocaine the depressor effect becomes pressor once more (Wyman and Tum Suden, 1935). This effect of cocaine resembles its effect in the hare in which the mixed constrictor and dilator response to stimulation of the abdominal sympathetic is transformed by cocaine into a simple constrictor response (Bülbring and Burn, 1937). Wyman and Tum Suden (1936) have examined the action of adrenaline in the rat after excluding the splanchnic circulation, and found that the pressor effect of a dose such as 0.1γ is then reduced by about half. After the injection of ergotamine the action of adrenaline was reversed, and the depressor effect was not modified by excluding the splanchnics. From this they concluded that the rat has more vasoconstrictor fibres in the splanchnic area than in the system of muscles and skin, but that vasodilator fibres are uniformly distributed in both systems. A comment on this deduction will be made later. Clark (1934) has shown that in the cat, intra-arterial injections of adrenaline (0.05γ) cause an increased outflow from the veins of the leg muscles.

The vasodilator action of adrenaline in the skin. The action of small doses of adrenaline in the skin vessels appears to differ according to the species. Clark (1930, 1934) examined the effect of intra-arterial injections on the outflow from the skin veins of the cat under chloralose, and observed constriction only; after giving ergotoxine there was no effect at all. In dogs under ether, on the other hand, Hoskins, Gunning and Berry (1916) found that the intravenous injection of adrenaline caused an initial increase of outflow from the skin veins followed by a

constriction. Bülbring and Burn (1936b) also recorded an increased outflow from the saphena vein in dogs under ether. There is, however, in the dog a sympathetic constrictor innervation of the veins, as first demonstrated by Bancroft (1898), and the increased outflow may have been due to venous constriction rather than to arterial dilatation; this is the more likely since all doses of adrenaline failed to increase the outflow after the administration of ergotoxine.

The vasodilator action of adrenaline in the intestines. The investigation of vascular changes in the intestine is more difficult than in the muscles or skin because the vessels seem to be readily damaged by exposure and handling of the intestines. Changes in volume due to changes in blood pressure are less easy to avoid; for these reasons observations made by Hoskins and Gunning (1917) and Hartman and Fraser (1917) appear unreliable. Clark (1934) working on cats, and recording the outflow from an intestinal vein, found that small doses of adrenaline injected intra-arterially always caused constriction. After giving ergotoxine, however, he observed vasodilatation. Bülbring and Burn (1936) working on dogs regularly obtained expansion of an intestinal loop in a plethysmograph when adrenaline was injected either intravenously or intra-arterially, and a similar expansion after the administration of ergotoxine. When loops of intestine were perfused with defibrinated blood, small doses of adrenaline injected into the artery caused constriction followed by dilatation, and after ergotoxine was given, simple dilatation. These effects in perfused loops were, however, small.

The vascular effect of adrenaline in active muscles. Rein and Schneider (1931) injected adrenaline into the veins of dogs under chloralose anesthesia and examined its effect on the blood flow through the femoral artery to the gastrocnemius muscle while the muscle was resting and while it was contracting. The blood flow was measured by the thermostromuhr. During rest adrenaline produced initial increase of flow followed by a diminution of flow; during muscular contraction when the blood flow was increased adrenaline produced a slight increase of flow only. Similar results were published in 1936 by Mertens, Rein and Valdecasas; they used dogs anesthetised with Pernocton and measured the flow in the femoral vein (below the entrance of the saphena vein).

The authors concluded that adrenaline injected intravenously is always inactive during the increased blood flow of muscular contraction and regarded this as an important modification of the action of adrenaline produced by muscular activity.

Bülbring and Burn (1938) have recently made similar investigations by perfusing the muscles of the dog's hind limb with defibrinated blood by a Dale-Schuster pump, stimulating the motor roots to produce contraction and recording the tension with a tension lever. When adrenaline was injected intra-arterially in doses from 2 to 5γ, they found that the inflow was diminished during a prolonged tetanus as it was during rest; the diminution was not so great as during rest, but there was no disappearance of the constrictor action as described by Rein and his colleagues. The disagreement between the two observations may be explained by the difference in the amounts of adrenaline reaching the muscle vessels. Since Rein and his colleagues injected 5γ adrenaline intravenously, while Bülbring and Burn injected the same dose intra-arterially, much less adrenaline would reach the muscle vessels in the experiments of Rein; if the effect of this amount was slightly reduced during muscular contraction, it might appear that there was no effect at all and so give the impression of a qualitative change in the muscle behaviour.

Sympathetic vasodilator fibres and histamine. In their paper on the vasodilator action of histamine, Dale and Richards (1918) showed that the effect of small doses on the hindlimb volume of cats under ether was always similar to the effect of small doses of adrenaline. In the limb normally innervated, histamine had little or no dilator effect, but after denervation the action was usually dilator. Dale and Richards found that whatever the response to histamine, the response to small doses of adrenaline was qualitatively the same. The similarity in this vascular inhibitor action has a parallel in the inhibitor action of histamine and adrenaline on the rat's uterus and in the dilator action of both substances in the coronary vessels of the cat.

The dilator action of histamine in the limb of the cat, an action which, according to Dale and Richards, is exerted on the capillary bed, is greatly augmented by degeneration of the sympathetic fibres, as was first shown by Dale and Laidlaw (1911). This augmentation follows excision of the stellate ganglion very quickly, being sometimes evident within 30 minutes of the extirpation. Thus if sympathetic impulses are removed, the dilator action of histamine becomes greater, without any corresponding change in the dilator action of acetylcholine. The evidence points to the conclusion that the sympathetic innervation affects the capillaries in such a way as to reduce the dilator action of histamine, and it is therefore possible that the sympathetic innervation of the capillaries is dilator. If this is so the capillaries concerned must be chiefly muscular, in view of the work of Clark (1934) already mentioned.

Sympathetic vasodilator fibres in the leg of the dog. The recent development of knowledge of vasodilator fibres in the sympathetic supply has come in the main from the use of the dog instead of the cat. Although the original evidence for vasodilator fibres was obtained by Dastre and Morat in the dog, few workers have made observations on dogs until recent years. In 1932 Burn described the effect of stimulating the lumbar sympathetic chain of the dog when the hind limbs were perfused with defibrinated blood through the abdominal aorta. He found that in an ordinary perfusion by means of a Dale-Schuster pump (1928), there was a very low vascular tone, and the response to stimulation of the sympathetic chain was negligible. Burn (1932a) had previously found that the pressor effect of tyramine in these circumstances was very slight unless adrenaline was added steadily to the perfusing blood to create an initial tone, and he now observed that a similar addition of adrenaline increased the response to stimulation of the chain. This response was constrictor on occasions, but very often it was vasodilator. Powerful stimulation of short duration was almost always dilator in effect, while prolonged stimulation was constrictor. It was often possible to see an initial vasodilatation giving place to vasoconstriction; when the stimulus was arrested and the vasoconstriction passed off, a residual dilatation was again seen. Similar effects were not obtained in cats. These observations of vasodilator responses to the stimulation of the sympathetic chain in the dog were important because the effects were obtained without the use of ergotoxine, and because they indicated the existence of a generalised system of vasodilator fibres. The dilatation obtained by Dastre and Morat was regarded as a special effect of the sympathetic system in the bucco-facial region.

The existence of these vasodilator fibres in the dog was also demonstrated by Schneider (1934) who examined the blood flow through the femoral artery of dogs anesthetised with Pernocton; he used Rein's thermostromuhr. He found that when the abdominal sympathetic chain was stimulated there was an immediate increase of flow through the femoral artery, followed by a small but longer lasting decrease. When the stimulus was repeated several times, the dilator effect diminished. The illustrations in Schneider's paper furnish beautiful examples of the value of the thermostromuhr method.

In 1935 Bülbring and Burn showed that the sympathetic dilatation previously described by Burn was cholinergic and occurred in the muscles. In perfusion experiments with defibrinated blood, the dilator effects obtained in response to brief stimulation of the sympathetic chain were augmented after the addition of eserine and abolished after

the further addition of atropine. Constrictor effects were reversed by the addition of eserine. When the muscles were perfused with Ringer's solution containing eserine (1 in 400,000) the venous effluent collected during stimulation of the sympathetic chain caused contraction of the leech muscle. In dogs anesthetised with Pernocton records were taken of the volume of one hindlimb after removal of the skin. Stimulation of the corresponding sympathetic chain for short periods produced constriction of the limb before the injection of eserine, and dilatation afterwards. This dilatation was once more reversed to constriction by the injection of atropine. The initial constrictor effect of sympathetic stimulation was also changed to dilatation by the injection of ergotoxine. This dilatation was augmented by the injection of eserine and abolished by atropine; on the other hand the dilator effect of injections of adrenaline after ergotoxine was unaffected by atropine. The interesting observation was made that the initial constrictor effect of stimulation could also be reversed to dilatation without the use of either eserine or ergotoxine by the slow infusion into a vein of a solution of adrenaline (1 in 100,000). Once more the dilator effect obtained was augmented by eserine and abolished by atropine. The infusion of pituitary (posterior lobe) extract did not have the effect of the adrenaline infusion. None of these effects obtained in the skinned leg of a dog under Pernocton anesthesia could be obtained in the cat. It was possible to obtain vasodilatation in the cat by stimulating the sympathetic chain after giving ergotoxine, but this vasodilatation was unaffected by eserine and atropine.

Bülbring and Burn discussed the reason for the change of the dilator mechanism from adrenergic in the cat to cholinergic in the dog. They pointed out that efficient working of vasodilator fibres will be of most importance to the body in times of emergency, but that at these times there is an output of adrenaline from the suprarenal glands which creates a high vascular tone, and that the transmitter liberated by the dilator fibres must exert its effect in opposition to this adrenaline tone. Now small doses of adrenaline have no vasodilator effect whatever in the perfused vessels of the dog's muscles when the tone is maintained by adrenaline, but the vasodilator effect of small doses of acetylcholine is actually increased by an adrenaline tone. The cholinergic dilator mechanism is therefore much more efficient.

Rosenblueth and Cannon (1935) observed that after giving ergotoxine, stimulation of the abdominal sympathetic in the dog caused a fall of blood pressure which was increased by eserine and abolished by atro-

þine; they expressed doubt whether the difference between the dog and the cat was really a qualitative difference, and thought that there were cholinergic fibres in the cat also.

The Sherrington phenomenon. Bülbring and Burn (1936a) brought evidence for the presence of a few cholinergic fibres in the cat by investigation of the Sherrington phenomenon. This consists in contraction of the leg muscles when the sciatic nerve is stimulated after first cutting the motor roots and allowing the motor fibres to degenerate. The contraction was long thought due to stimulation of the sensory fibres in the sciatic, but Hinsey and Cutting (1933) found that it still occurred after degeneration of the sensory fibres, and concluded it must be due to sympathetic fibres; in the cat, however, they obtained an effect once only on stimulation of the grey rami, and never by stimulation of the chain. If the effect was due to acetylcholine liberated from sympathetic fibres, it should be readily obtained in the dog. Bülbring and Burn found that this was so, and were able to obtain very feeble effects in the cat also when the chain was stimulated.

The dilator supply to the muscles of the hare. The observation that the sympathetic chain of the dog contained many vasodilator fibres, whereas that of the cat contained very few, suggested that this innervation might be related to the dog's greater capacity for prolonged exertion. An examination was therefore made of the sympathetic chain in the wild hare (Bülbring and Burn, 1937). Plethysmograph records were obtained of the skinned hind limb under Pernocton anesthesia; they showed that stimulation of the sympathetic chain caused constriction while the stimulus was applied, but prompt vasodilatation when the stimulation stopped. For stimuli of short duration the vasodilatation was greater than the constriction, but it did not last very long. The constrictor effect could not be separated from the dilator effect, for after the injection of either ergotoxine or cocaine, the dilator effect disappeared; after giving ergotoxine the constrictor effect also was reduced. These results in the hare were compared with results obtained with wild rabbits caught on the same land; in the rabbits no vasodilator effects were seen after stimulation. When vasodilator substances were injected, the difference between the hares and the rabbits was very striking; with acetylcholine, adenosine and adrenaline, excellent dilator effects were obtained in the muscles of the hare, but almost no dilatation in the muscles of the rabbit.

The innervation of the muscles of the monkey. The monkey (Macacus rhesus) is an animal which, unlike the dog, is rapidly fatigued. When

examined by the method described, it was found to have no vasodilator fibres in the sympathetic chain. Even after large doses of ergotoxine, which reversed the action of adrenaline so that it became depressor and vasodilator, sympathetic stimulation still produced vasoconstriction. Further it was found that acetylcholine and adenosine had little or no dilator action. Histamine, however, which is pressor in the hare as in the rabbit, had a good dilator action in the monkey.

The action of vasodilator fibres in active muscle. In the five species rabbit, monkey, cat, hare and dog there are readily observed differences which afford useful indication of the importance of sympathetic vasodilator fibres to the vessels of the muscles. The fibres are absent in the rabbit and the monkey, demonstrable only with difficulty in the cat, and at once evident without the use of drugs like ergotoxine or eserine in the hare and in the dog. In the cat it is the depressor effect of adrenaline which is easily seen after giving ergotoxine, and not the vasodilator effect of sympathetic stimulation; this effect is relatively small. The effect of adrenaline after ergotoxine is not a good guide to the existence of vasodilator fibres, as different workers often assume (cf. Wyman and Tum Suden, 1936), since in the monkey the adrenaline reversal can be obtained, while the effect of sympathetic stimulation is always constrictor.

In order to discuss the part played by vasodilator fibres in contracting muscles, it is necessary to say something about the Orbeli phenomenon (1923). Orbeli showed that when rhythmic stimulation was applied to the anterior roots in the frog, the contractions of the muscles became less due to fatigue, but augmented if the sympathetic chain was stimulated. Baetjer (1930) obtained similar results in the cat, though she also found that sometimes the opposite effect followed sympathetic stimulation, and the contractions of the muscles were diminished. Bülbring and Burn (1938) have worked on dogs, making observations in dogs anesthetised with Pernocton, on spinal dogs and in dogs' muscles perfused with blood by the Dale-Schuster pump. They have observed, in confirmation of Baetjer, that sometimes stimulation of the sympathetic chain produces an increased muscle tension during a prolonged tetanus, and sometimes a decreased tension. Evidently the effect of stimulation of the sympathetic chain, so far as the vasoconstrictors are concerned, is twofold. The vasoconstriction diminishes the blood supply to the muscle and so diminishes muscle tension; on the other hand the humoral transmitter liberated from the vasoconstrictor endings diffuses into the muscle substance and by its effect on muscle

metabolism increases the muscle tension. Either of these two effects on the tension may predominate according to the conditions of the experiment, or they may cancel each other.

The part played by the vasodilator fibres in this mechanism has not yet been discovered. Bülbring and Burn have been able to show that brief stimulation of the sympathetic chain causes vasodilatation during a prolonged tetanus just as it does during rest, but they have not yet been able to demonstrate the effect of this vasodilatation upon the muscle tension. When we remember that these fibres are cholinergic in the dog, it is reasonable to suppose that they will be found to improve the efficiency of muscular contraction still further.

Vasodilator fibres in the intestines. After the injection of ergotoxine, Bülbring and Burn (1936b) were able to observe dilatation in intestinal loops in cats when they stimulated the splanchnic nerve, having first removed both suprarenal glands. These dilator effects were observed by taking plethysmograph records and were small. Much larger dilatations were obtained when the same procedure was followed in dogs. In one experiment in a dog, the infusion of adrenaline into the jugular vein reversed the constrictor effect of brief splanchnic stimulation, so that dilatation was consistently obtained without the injection of ergotoxine. Dilatations in intestinal loops were not augmented by eserine or abolished by atropine, and indeed it was observed that acetylcholine itself had no dilator effect. Small dilatations were observed when the splanchnic nerve was stimulated during the perfusion of the intestinal vessels of the dog with defibrinated blood by means of the Dale-Schuster pump.

The number of vasodilator fibres in the dog's splanchnic nerve is evidently much less than that in the abdominal sympathetic chain, and it is more difficult to understand the purpose of these fibres in the splanchnic supply. Probably they innervate the peripheral branches of the arteries, so that while constriction of the larger arteries safeguards the general blood pressure, dilatation in the terminal arterioles or capillaries facilitates the blood flow to the intestines.

Vasodilator fibres in the skin. Sympathetic vasodilator fibres in the skin have a special interest not shared by those of the muscles and intestines, in that the observations in animals can be compared with those in the human subject. As already described, Clark (1934) was unable to observe any dilator action of small doses of adrenaline injected intra-arterially in the cat, either before or after giving ergotoxine. Bülbring and Burn (1936b) observed an acceleration of venous outflow in the

dog, but since this disappeared after giving ergotoxine it was possibly due to venous constriction. Cotton, Slade and Lewis (1916) found that the injection of 1/30,000 adrenaline under the skin of the human subject produced intense blanching even after occluding the vessels of the upper arm. They say that the blanching must be due to capillary constriction and not to a lack of blood supply to the capillaries. Thus the evidence which can be obtained from the action of adrenaline indicates that in the cat, dog and human subject, there are no adrenergic sympathetic vasodilator fibres.

Bülbring and Burn (1936b) investigated the effect of stimulating the lumbar sympathetic chain on the outflow from the saphena vein in the dog, and observed an initial acceleration which then gave place to a slower outflow. When they perfused the skin vessels of the hind limb with defibrinated blood, recording both inflow and outflow, they found that stimulation of the chain caused diminished inflow, but the effect on the outflow was the same as in the whole animal, namely, initial increase and later diminution. After giving ergotoxine there was no effect on either inflow or outflow. These results can best be explained by supposing that when the sympathetic chain is stimulated, the skin arteries constrict and so diminish the inflow; the skin veins also constrict and cause an initial increase of outflow; thereafter the outflow diminishes because of the diminished inflow. The constrictor effect on the veins, like that on the arteries, is paralysed by ergotoxine.

When the hind leg of the dog is placed in a plethysmograph the large constriction which occurs on stimulation of the sympathetic chain is chiefly in the skin; about 20 per cent only is due to vasoconstriction in the muscles. This large constriction in the skin is in part due to diminished inflow, but also to the venous constriction which drives into the general circulation a large amount of blood. After the injection of ergotoxine, stimulation of the sympathetic chain causes dilatation of the leg, but the whole of this dilatation is abolished by atropine, so that it is a dilatation caused by acetylcholine. Now acetylcholine has no dilator action on the vessels of the dog's skin, so that the whole of the dilatation caused by sympathetic stimulation must be in the muscles.

Although Bülbring and Burn thus failed to obtain evidence of sympathetic vasodilator fibres in the skin of the dog's hind limb, they observed their presence in the dog's ear. They took a plethysmograph record of the ear volume, and observed the effect of stimulating the cervical sympathetic chain after the injection of ergotoxine. Vasodilatation was seen which was unaffected by the administration of atropine.

Presumably this vasodilator supply is a mechanism concerned with heat regulation.

Observations in human skin. While evidence of a sympathetic vasodilator supply to the skin of the cat and dog is lacking, Lewis and Pickering (1931) have brought forward evidence for the existence of such a supply in the human subject. They observed that when a patient, whose left arm had been deprived of its sympathetic nerves, was introduced into a chamber while his hands remained outside, and the chamber was then heated, the temperature of his normal hand rose, but the temperature of the hand without sympathetic fibres remained unchanged. Similar observations were made in normal subjects kept at room temperature by anesthetising the ulnar nerve of one side at the elbow, and recording the temperature of the skin of the corresponding 5th finger. When the anesthesia was complete the temperature of the anesthetised area rose, evidently due to the removal of vasoconstrictor impulses. Now the curious observation was made that if patients suffering from Raynaud's disease were exposed in a cold room until the fingers were fully cyanotic, the temperature of the 5th finger could not usually be raised by anesthetising the ulnar nerve, although the finger could be reddened. The warming of the body of the patients, while their hands remained exposed to cold was, however, followed by a rise of temperature of the unanesthetised fingers. Lewis and Pickering argued that this rise must have been due to active vasodilatation and not to the removal of constrictor tone because of the results in a patient with Raynaud's disease in whom the ulnar nerve was first anesthetised and who was then placed in the warm chamber. The anesthetisation did not affect the skin temperature of the corresponding 5th finger, and when the patient was placed in the warm chamber the skin temperature still remained unaffected, although the skin temperature of the unanesthetised area rose. At that point of the experiment the skin temperature of the 2nd finger of the hand was 32.5°C., while that of the 5th finger was 15.8°C. The failure of the temperature of the anesthetised skin to rise when the body was warmed was, therefore, due to the interruption by the anesthetic of active dilator impulses.

Uprus, Gaylor and Carmichael (1936) however, do not appear to be satisfied that the conclusion drawn by Lewis and Pickering was sound. Although at one point of the experiment just described the temperature of the anesthetised finger was much lower than that of the normal finger, later the temperature of the anesthetised finger rose to that of the normal finger, so that the difference between the two was

really a difference in rate of rise of temperature. Carmichael and his colleagues find that the rate of rise of temperature depends on the local temperature of a limb, on the posture and on the rapidity of rise of blood temperature. There may be vasodilatation in the skin of one leg with no change in the skin of the other, and vasodilatation in one hand may be much delayed after that in the other. They think that the hot air bath is not a suitable method of producing dilatation, since it does not cause a steep rise of blood temperature, and that the time of onset of vasodilatation in limbs of a subject whose blood temperature is not rising rapidly should not be accepted as an indication of disturbance of the nervous mechanism.

The existence of sympathetic vasodilator fibres in human skin (of the hands and feet at least) must, therefore, be regarded as still doubtful.

SUMMARY

Until five years ago evidence for the existence of sympathetic vasodilator fibres had not advanced much beyond the point where it was left by Dale's demonstration in 1906 that after giving ergotoxine some of the motor effects of sympathetic stimulation were reversed. There was a general belief in the possibility of vasodilator fibres, but evidence obtained from the use of ergotoxine was not accepted by everyone. Recent work has shown that there are important differences in different parts of the body. In the muscles of the dog are cholinergic vasodilators in the sympathetic supply, easily demonstrated without ergotoxine. In the skin of the dog there are no sympathetic vasodilators except in the skin of the ear. In the intestines there are some vasodilators, presumably adrenergic, only to be demonstrated after giving ergotoxine. There are important differences in different species. In the rabbit and the monkey there are no sympathetic vasodilators to the muscles; in the cat there are a few, while in the dog and the hare there are many. The effect of stimulating the sympathetic supply to the muscles of the hare is a curious mixture of constriction and dilatation; had our knowledge of the sympathetic control of the blood vessels been based on the hare instead of on the cat, current teaching might have been entirely different.

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THE SITES OF FORMATION OF ESTROGENIC SUBSTANCES IN THE ANIMAL BODY

GEORGE W. CORNER

Department of Anatomy, The University of Rochester, School of Medicine and Dentistry

It is highly probable, though not conclusively demonstrated, that the estrogenic substance occurring in the mammalian female body is actually formed in the ovary. The evidence for this conclusion is simply that removal of the ovary promptly results in symptoms of hormone deprivation which are irreversible except by replacement by ovarian grafts or the injection of estrogenic substances. These symptoms are 1, castrate atrophy of the reproductive tract, readily curable by injection of estrogenic substances, and 2, abolition of reproductive cycles, some features of which are again restored by suitably timed administration of estrogenic hormone.

Search for depots of estrogenic hormone in the body began of course with the ovary, where significant amounts are found. It must be admitted that the ovary does not contain the only store of the estrogenic substances, yet most of the other observed sites can be explained without giving up the assumption that production takes place in the ovary. Presence of such substances in the blood and urine is explainable, for example, on the basis of transport and excretion. Presence in the mammary glands and in milk (Brouha and Simonnet, 1928; Tchajkovsky, Guil and Kousnetzova, 1936), in the non-pregnant uterus (Frank, Goldberger, Salmon and Friedman, 1935) and in the sexual skin of monkeys (Fisher, Krohn, and Zuckerman, 1936) might be explained by assuming that in these situations the hormone has been accumulated in the process of acting upon the tissues in question. Small amounts of an unidentified estrogen have been found in the bile (Gsell-Busse, 1928a, 1928b, 1929). It is not impossible that owing to the special rôle of the liver in sterol excretion, the ovarian steroids are (because of their chemical resemblance to cholesterol) subject to excretion by the liver. This in turn perhaps offers sufficient explanation of the presence of the hormone in the feces (Dohrn and Faure, 1928).

Estrogenic substance has been found also in extracts of the adrenal

cortex (Callow and Parkes, 1936, confirming Engelhart, 1930) and in extracts of the hypophyses of cattle (same authors; loc. cit., confirming Brouha and Simonnet, 1927a). Pending identification of the estrogen, Callow and Parkes offer no conjectures as to the significance of its presence in the adrenal, except the tentative suggestion that it may be merely a by-product of the formation of other steroids concerned in the function of the adrenal cortex. No explanation is available for the presence of estrogenic substance in the human saliva during pregnancy (Trancu-Rainer, 1931).

The problem becomes still more complex when evidence is brought forward that relatively large amounts can be extracted from the muscles of human females at autopsy (Frank, Goldberger, Salmon and Friedman, 1935) and that estrogenic hormones can be found in the blood and urine after castration and the menopause (Frank, Goldberger and Salmon, 1936). Frank and his co-workers do not assume that there are extra-ovarian sources of the hormone, but rather that there are extra-ovarian storage places. Other explanations, however far-fetched, suggest themselves. Is it possible that the ovary itself conducts merely intermediary or terminal stages of preparation of the hormone? Or are there substances, present in muscle and other extra-ovarian sites, which give the standard vaginal smear test and yet are not true estrogenic hormones capable of taking part in cyclic changes or of preventing castrate and post-menopausal atrophy? It has been shown recently that a whole series of chemical compounds have the property of causing vaginal smears of estrous type. A table of such substances is given by Dodds and Lawson (1936), and Robson and Schönberg (1937) have added another potent in 1 mgm. dosage. Evaluation of the significance of hormone-like substances found in various tissues will obviously require actual chemical identification.

Meanwhile we always come back to the fact that total removal of the ovaries causes the specific symptoms of hormone deprivation. Whatever the other stores or sources of estrin may signify they cannot replace the ovary. All the writers have assumed, therefore, that the ovary is the actual site of estrin production, not merely the controller or storage-place, and we may therefore proceed to examine in detail the further evidence as to what individual tissues may be involved in this function.

The diagram (fig. 1), a slightly conventionalized representation of the mammalian ovary, will serve to identify the various tissues and structures we shall need to mention. 1. The *follicles* comprise three

elements, namely the follicular fluid, the lining of granulosa cells and the subjacent theca interna. In the mature ovary, follicles are present in all phases of elaboration from the very simple primordial stage, in which the whole structure is composed of a single layer of future granulosa cells closely surrounding the ovum, to a stage in which the wall is differentiated into layers and there is a relatively large cavity. At intervals of time determined by the cycle of the species, a crop of follicles (or a single follicle, in animals bearing only one embryo at a time) proceeds rapidly to full maturity. During this final phase, which

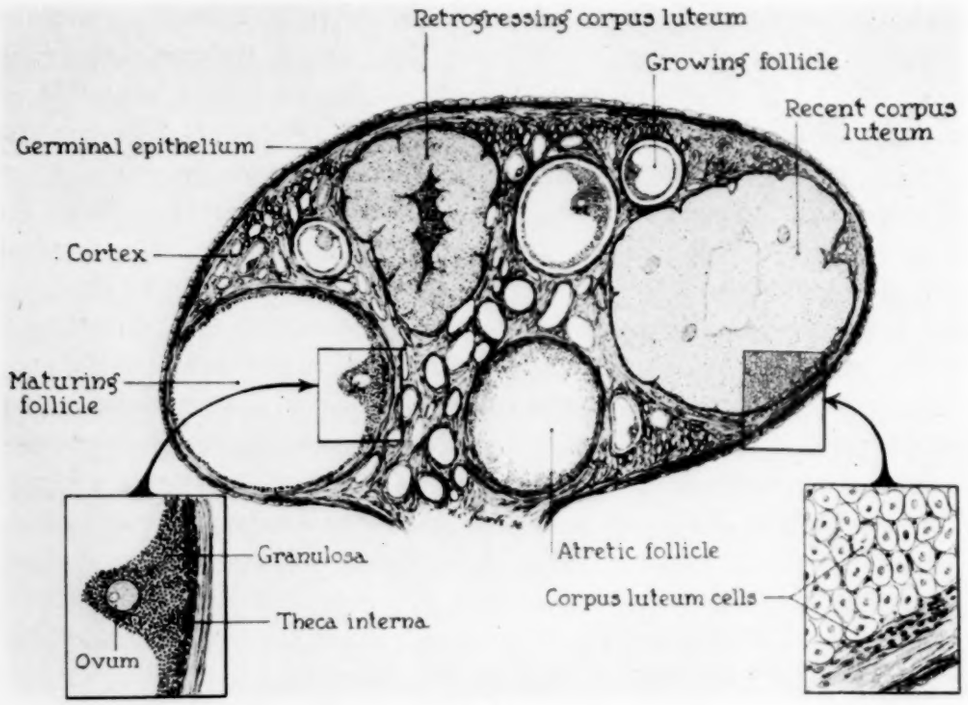


Fig. 1

lasts only a few hours or days, the size of the follicle, and consequently the amount of follicular fluid, increases several fold and the cells of the theca interna undergo change from fibroblastic to epithelioid aspect.

A majority of the follicles do not attain maturity, but undergo destruction by atresia. This process differs considerably in the various species, but has as a general characteristic the persistence and often the proliferation of the theca interna.

In a few species the granulosa cells also persist, and become epithelioid, but in most animals the granulosa is broken down. In the sexually mature animal atretic follicles are usually present, and sometimes a

large proportion of all the follicles which have grown sufficiently to possess conspicuous cavities have become atretic.

2. The *corpora lutea*, formed from those follicles which reach full maturity and shed their ova, are in most species (and probably in all) composed of two types of cells, derived respectively from the granulosa and the theca interna, the former being more numerous.

3. In many species the stroma of the ovary contains *interstitial cells* (not indicated in the diagram). These lipid-laden epithelioid cells occur in clumps or even, as in the rabbit, pervade the entire stroma and fill up all the space not occupied by follicles, corpora lutea and blood-vessels. It is fairly clear that the interstitial cells are derived from the theca interna of atretic follicles, and probably also by modification of the cells of the stroma and of the various epithelial proliferations in the earlier development of the ovary. In the human ovary there are no distinct groups of interstitial cells, but there are a few lipid-laden cells in and about the remains of atretic follicles. It may be that these cells, which look alike, whether found in great masses in the stroma or in thin layers in atretic follicles, are physiologically the same. For a more complete discussion see Corner (1932).

I. Estrogenic hormone in the follicle. When Allen and Doisy began the work which led to the isolation and chemical identification of the estrogens (cf. Allen, Doisy et al., 1924) their first source of an active extract was the follicular fluid. It is not surprising therefore that of all the elements of the ovary, the follicle was considered to be the actual source of the hormone.

The hypothesis that estrous phenomena are due to a secretion of the follicle, rather than of some other element of the ovary, was already widely, if somewhat vaguely, entertained, because observations on various mammals had made it clear that the mature phase of the follicles is closely associated with the phenomena of estrus. Loeb and Hesselberg (1917), for example, stated that some of the cyclic changes in the uterus must be due to a secretion of the follicles.

Robinson (1918) expressed the hypothesis very clearly. This author made a very careful morphological study of the ovarian follicles in ferrets and ferret-polecat hybrids, animals in which the cyclic phenomena of reproduction are well adapted to permit comparison of physiological states with ovarian histology. Robinson was so struck by the coincidence of follicular maturation with the estrous state, that he wrote ". . . the follicles at a certain period of their history appear to take on the function of providing the secretion which is responsible for

the phenomena of the pro-oestrum and the oestrus . . . Oestrus occurs only when the ovaries contain follicles in a certain stage of development, and such follicles are present only as long as oestrus continues. Under these circumstances it can scarcely be doubted that the phenomena of heat are due to something produced by the follicles." Readers interested in the details of experiment and conjecture in the pre-estrin era will find a review and references in Robinson's monograph.

Hartman (1923) observed a similar correlation between follicular maturity and estrus in the opossum, and stated his complete adherence to the views of Allen and of Robinson. As the cyclic changes in the ovaries of mammals have become more generally known, it has become generally obvious that estrous behavior and estrous changes in the vagina and other parts of the reproductive canal are associated (in point of time, at least) with maturity of the ovarian follicles. The only known exception is in certain bats (Courrier, 1922, 1928; see below).

In view of this evidence pointing so strongly to the follicle as the source of estrogen, Allen and Doisy proceeded to name one of the layers of the follicular wall, namely, the membrana granulosa, as responsible for production of the hormone. In this conjecture they were guided apparently by the simple fact that the granulosa is the most conspicuous layer. Robinson (1918) had ascribed his conjectural follicle hormone to the granulosa. Allen and Doisy found also that the solid ovarian tissue, after the follicular fluid had been aspirated from all large follicles, yielded potent extracts, tentatively explained as coming from the granulosa layer left in situ in the follicles after the fluid was withdrawn.

The argument was reinforced by experiments of Allen, Pratt, Newell, and Bland (1930), in which pieces of human ovarian tissue were implanted subcutaneously into young spayed rats, the estrogenic effect being observed by examination of the vaginal contents. When the implanted tissue consisted of cortical stroma without visible follicles, the results were negative, even though the amount tested was 0.89 to 2.65 grams. On the other hand, pieces of the walls of large follicles, said to consist mostly of granulosa cells, yielded up to 5 rat units of hormone per gram, the fluid of the same follicle having yielded 3 rat units per cubic centimeter.[1]

[1] Owing to variations in methods, standards, and species of animals used in vaginal smear test by different authors, it is not advisable to convert the dosages or yields to a common standard. The reviewer has therefore quoted quantities of hormone in the terms used by the original author. For rough comparison the reader may assume that 1 International Unit = 1 mouse unit = 0.2 rat unit.

Contrary to this first view that the granulosa is the seat of estrin production are some qualitative experiments of Aschheim (1926), who implanted bits of human ovarian tissue into spayed mice. Cortex without follicles was negative as to the production of estrous smears, but cortex containing atretic follicles was positive. Granulosa scraped from the wall of a large follicle was negative. The theca wall was positive. The authors concluded that the theca cells are the site of production of estrin.

It will be seen that Aschheim agreed with Allen and Doisy in thinking that the follicle is the source of estrogenic hormone, differing only about the particular layer of the follicular wall considered to be responsible. We turn next to observations which raised a question as to whether the follicles are concerned primarily, or at all, in this function. Courrier (1922, 1928) pointed out that in a bat (Vesperugo pipistrella) estrus and mating occur in September of each year, although the follicle does not mature until much later, after hibernation.

Dickens, Dodds, and Wright (1925) attacked the problem of the site of hormone production in another way by differential extraction and comparison of hormone yield from follicular fluid versus that from the solid ovarian tissue. The yields were of the same order. This result did not support the contention that the active principle is mainly confined to the liquor folliculi. Parkes and Bellerby (1926) used frozen ovaries of cow, pig, sheep, and mare. By dissection in the frozen state the solidified follicular content was separated from the ovarian substance, and the two tissues were extracted separately. In eight batches, the yield of mouse units per kilogram of follicle fluid was higher than that of residual tissue three times, lower five times. Parkes and Bellerby also concluded from this that the pre-eminent importance of the follicle as source of the hormone had been over-emphasized.

This conclusion was drawn again from experiments of a different kind. Parkes (1926–1927) while engaged in the examination of mice which had been treated with x-rays when three weeks of age, came upon an animal in which the uterus and vaginal contents were in typical estrous condition, although the ovary had been so greatly injured by the rays that all the follicles had been destroyed. Following this clue, he observed the estrous cycles (by examination of vaginal smears) in mice similarly treated, and found a number of animals in which cyclic recurrence of estrous changes took place. The cycles were never, it must be admitted, perfectly regular and of normal length. The best

animal exhibited three cycles of average duration, slightly over 7 days. (The normal cycle-length in the mouse is 4 to 6 days.) In many of the estrous periods of irradiated mice, copulation occurred when opportunity was given; this is a critical test of the estrous condition. Histological examination of the ovaries showed that the oöcytes, granulosa cells and theca cells were caused to degenerate and disappear, and the ovarian stroma was invaded and ultimately filled by a mass of tissue originating from the germinal epithelium. This tissue was composed of epithelioid cells arranged in cords surrounded by slight fibrous sheaths.

These findings, as Parkes remarked, "lead inevitably to the conclusion that in the mouse, at any rate, the phenomena of oestrus may occur quite independently of the presence of any follicles." He seems to have taken it for granted that the newly formed ovarian tissue is not to be compared with any element of the normal ovary except the interfollicular tissue, and he therefore concluded that the interfollicular tissue is normally the source or one of the sources of estrogenic hormone. Naturally his experiments did not exclude the possibility that the follicles, when present, may also contain hormone-producing cells, but on the whole this demonstration that the follicles are not essential seemed at the time a radical departure.

When the ovaries of adult mice were irradiated, similar destruction occurred (although not so completely). The epithelioid tissue which finally filled up the ovary in large part was found to be derived by hypertrophy of various ovarian elements, including according to Parkes the theca interna cells, the granulosa, and interfollicular tissue.

A valuable continuation of this line of study is that of Ida Genther Schmidt (Genther, 1931, 1934; Schmidt, 1936) who irradiated the ovaries of adult guinea pigs and thereafter followed not only the estrous behavior and vaginal contents but also the excretion of estrin in the urine. She found that suitable treatment with x-rays caused the conversion of the ovaries into masses of epithelioid cells, identified with the interstitial tissue because of their histological resemblance to the so-called "interstitial gland," as the extensive masses of such tissue occurring in the rabbit have often been called. Abolition of follicular development was not however complete, for an occasional follicle could often be found. Animals in which the histological change was most nearly complete showed very irregular recurrence of estrous vaginal smears, with prolonged periods of opening of the vaginal closure membrane. During some part of each of these phases there was excretion

of estrin in the urine, in quantity usually but not always less than that found in urine of normal guinea pigs during estrus. The known occurrence of a follicle or two in temporal relationship to these irregular estrous periods complicates analysis of the findings, but Schmidt is of opinion that the follicular tissue is not sufficient in quantity to account for the production of the estrin and that the cells of the tissue resulting from the irradiation must be the chief source of the hormone.

These experiments with x-rayed ovaries, especially the earlier work of Parkes, raised an apparent difficulty. In normal animals the action of estrin, and apparently its very presence, is cyclic, a fact which was easy to explain if the follicles, themselves cyclic in development, were the source of the hormone. The interfollicular tissue to which Parkes and Schmidt ascribe most or all of the hormone production does not come and go cyclically.

This difficulty is removed, however, by considerations based on the relations between the ovary and the hypophysis. At present the estrous cycle is best explained by a hypothesis (current in several variant forms; for review see Corner, 1933) involving alternate phases, in one of which the hypophysis stimulates the ovary to produce estrin, in the other the estrin in turn depresses the pituitary gonad-stimulating action, and so on repeatedly. In such a scheme it is not necessary to postulate actual appearance and disappearance of the estrin-producing tissue, but merely quantitative alternations of functional activity.

A further clarification is suggested by the embryology of the ovary. Not only are the elements of the follicular wall (granulosa cells and theca interna) derived ultimately from the mesoderm of the embryo, but so also are whatever epithelioid cells may be present in the stroma, whether occurring as an "interstitial gland" (e.g., rabbit) or as sparse and ill-defined groups (human). The atypical cell masses which fill the ovary after treatment with x-rays are likewise derived directly from the germinal epithelium (in immature animals) or indirectly (in mature animals), and hence ultimately from mesoderm. These various cell-types, being related, may be so much alike that it is often difficult and in many cases impossible to distinguish one from another, except when the cell in question is clearly seen in place in one of the formed structures, i.e., the follicle, a definite island of interstitial cells, or the corpus luteum. There is for example no cytological criterion for distinguishing theca interna cells from the interstitial cells; and even the granulosa cell and its derivative, the granulosa lutein cell, may when its period of specialized function is over, that is, in late pregnancy and in retrogressing

corpora lutea, come to look much like the theca cell. For example, in one of the South American monkeys which happens to have an extensive "interstitial gland" filling the ovarian stroma, it happens also that the granulosa lutein cells of the corpora lutea of pregnancy look exactly like the interstitial cells, and furthermore the theca externa around the corpora lutea is very slight and inconspicuous. Thus the corpora lutea, though present, are not distinguishable (personal communication of Dr. F. F. Snyder). Not even an expert can distinguish theca interna cells from corpus luteum or from interstitial cells, in this animal.

From such considerations it appears altogether likely that the cell-masses which appear from the experiments of Parkes and Genther-Schmidt to form estrin, are genetically and functionally similar to one or another of the usual epithelioid elements of the ovary. The experiments with x-rays have demonstrated that organization of the cells into follicles is not necessary for the production of estrin; but they have in no wise excluded the possibility that in the normal ovary estrin-producing cells may be present in the follicular wall.

Renewed evidence of the production of estrin in the wall of the growing follicle is provided by the ingenious experiments of Westman (1934). This investigator states that he is able to destroy the ovarian cortex of a rabbit with a fine diathermy needle, so accurately that he can leave intact a single small or large follicle. Experiments were done in the following order.

1. Immediately after mating the cortex was destroyed, leaving one ripe follicle in one ovary, the other ovary being removed. The surviving follicle practically never became a normal functioning corpus luteum.

2. The same experiment was done 1 to 5 days after mating (before implantation), leaving 1 corpus luteum. The result was disturbance of the development and hormonic function of the surviving corpus luteum. The corpus luteum cells remained small and acquired a pale staining reaction, and there were only slight signs of progestational response of the endometrium.

3. The same experiment was done from the 7th to the 24th day of pregnancy, leaving 1 corpus luteum. The result was degeneration of the corpus and resorption of the embryos.

4. In the unmated rabbit the ovarian cortex was destroyed, leaving one growing follicle. The other ovary was extirpated as usual, and the animal was given an injection of gonadotropic hormone (powdered hypophysis) in order to cause ovulation. The result was development

of the follicle into an incomplete or complete corpus luteum without hormone production.

5. In order to test the possibility that the operative procedure had caused a general disturbance of the tissues or of the circulation, experiments were done in such a way that a number of growing follicles remained protected from destruction in one ovary. In others the entire cortex of one ovary containing the growing follicles was completely destroyed, while the other ovary was left in place, its ripe follicles or corpora lutea having been destroyed while the growing follicles were protected. These experiments were done from the day of mating up to the 20th day afterward. The results showed that a ripe follicle can develop into a normal functional corpus luteum if a number of growing follicles are present. Experiments done when active corpora lutea were present in the ovary showed that when the follicles in both ovaries were incompletely destroyed or when the follicles of one ovary were completely destroyed while follicles in the other were protected, the corpus luteum remained undisturbed.

All these experimental results, taken together, were interpreted to mean that the developing follicles produce a hormone which is necessary to the development and maintenance of the corpora lutea.

6. Acting upon the supposition that the suspected hormone is estrin, a series was done in which the cortex was destroyed, leaving one ripe follicle, and the animal was thereafter given estrin in large single injections (1000 to 3000 mouse units) at the time of operation. In four of these five experiments done immediately after mating, with administration of estrin at the same time, the surviving ripe follicle was converted into a functional corpus luteum. In similar experiments done later, the surviving corpora lutea remained functional. In five experiments in which progestin was given, the follicles or corpora did not survive.

7. In unmated rabbits similar operations were performed, leaving one growing follicle. The animals were then given pregnancy urine or a combination of estrin and powdered pituitary. In all but two of ten experiments the corpus luteum developed normally and became functional.

8. In nine experiments the cortex was destroyed, leaving 2 or 3 medium sized follicles. The granulosa of these follicles was removed by suction, leaving the theca (as demonstrated by subsequent microscopic study). A pituitary preparation was administered, but in five of the experiments this was not done at once but three days after the

operation. In the first sub-group in which the gonadotropic substance was given promptly the uterus showed what Westman calls an estrous reaction. In those in which the pituitary hormone was given late no estrous reaction occurred. This is interpreted to mean that the theca cells when stimulated by the pituitary become the source of estrin, while they are in relationship with functioning granulosa.

From the whole series of experiments Westman draws two chief conclusions: 1, development, persistence and function of the corpus luteum is dependent upon a supply of estrin; and 2, the theca cells are the seat of formation of estrin. The interstitial cells do not produce estrin. He states, however, that castration effects have been found to set in more rapidly after extirpation of the ovaries than after destruction of the cortex alone by burning. He interprets this to mean that the interstitial tissue is a storage place for estrin.

Mossman (1937) calls attention to instructive conditions existing in the pocket-gopher, Geomys bursarius. In this species the theca interna is very greatly developed, forming masses termed "the thecal gland," which reach their greatest extent at the time of estrus. This periodic increase of theca interna cells may be correlated with a high level of estrin secretion not only on the basis of general principles of the estrous cycle, but also because of a special phenomenon occurring in the gopher (and certain other rodents). At the first estrous period the symphysis pubis of the female is suddenly resorbed. Similar resorption can be produced in the castrate female by injections of estrin. It may well be, therefore, that the "thecal gland" of Geomys serves to produce the necessary estrin.

Another observation taken to show the source of estrogenic substances in the ovary has been developed on an experimental basis by Freed and Soskin (1937). When rats 6 to 20 days old were given gonadotropic hormone of pregnancy urine (APL) for 4 days and autopsied on the 5th day, the effects of treatment upon the ovaries and uteri were found to vary according to the age of the ovary. In younger animals the ovarian effect consisted in alterations of the theca interna and interstitial cells, which attained an epithelioid structure (in the words of the authors, became "luteinized"). In these animals the uterus had enlarged considerably, but the endometrium remained infantile. In the older animals (16 to 21 days) there were changes not only in theca cells and interstitial tissue of the ovaries, but also in the granulosa cells; in these the uterus was still larger and the endometrium had developed columnar epithelium. The possibility that the uterus in the younger animals was merely refractory to estrin was at least

partially excluded by the observation that in 14-day rats treated with estrone the endometrium responded fully. The authors' conclusion is that the rat's ovary elaborates two estrins, one "incomplete" secreted by the theca interna, the other "complete" secreted by the granulosa. They seem not to have considered at all the probability that the effect of the gonadotropic hormone upon the very immature ovary might bring about a preliminary state of morphological change, with only gradual development of hormone production, the result being not two hormones in succession, but merely a progressive effect of one hormone.

In summary, although the evidence from all these lines of investigation falls short of actual proof, taken together it indicates strongly that the usual site of production of the estrogenic hormone is the theca interna of the Graafian follicle. Hormone production probably begins when the follicle is small and continues as it grows large.

II. Estrogenic hormone in the corpus luteum. The finding of estrogenic hormone in the corpus luteum was foreshadowed by a number of early investigations made before the application of the vaginal smear test (e.g., Frank and Rosenbloom, 1915) in which the corpus luteum was found to yield extracts capable of preventing castrate atrophy of the uterus, and of stimulating engorgement and growth of the immature genital tract. In 1925 Frank and Gustavson showed that extracts of the corpora lutea of cow and sow give the vaginal reaction.

That the principal hormone of the corpus luteum, progesterone, is not responsible for the estrogenic effect was demonstrated by W. M. Allen and Meyer (1933) who succeeded in separating the estrogen chemically from the progesterone. The progesterone did not have an estrogenic effect upon the vagina. Korenchevsky and Hall (1937) obtained neither estrogenic action upon the vagina nor growth of the uterus in oöphorectomized rats, with 500γ of pure progesterone per day. The best assay of the amount of estrogenic hormone in the sow's corpora lutea, that of W. M. Allen and Meyer (loc. cit.) gave 60 to 80 rat units per kilogram of fresh tissue. The human corpus luteum contains a much larger amount of the hormone. Allen, Pratt, and Doisy (1925) obtained by extraction of excised corpora a yield of the order of 2 or 3 rat units per cubic centimeter (about 2000 to 3000 r.u. per kilogram) and in 1930 Allen, Pratt, Newell and Bland, testing human corpora by implantation of fragments into rats, had yields up to 16 rat units per gram (16000 per kilogram) from corpora of the non-pregnant subject. The yield appeared to be higher in the first days after ovulation. Corpora lutea of pregnancy generally gave smaller yields.

Since the corpus luteum contains, according to the prevalent view,

derivatives of both epithelioid layers of the follicular wall, there is no difficulty in finding cells to account for production of the hormone.

We can only guess as to the functional significance of the estrogenic substance in the corpus luteum. Evidence is accumulating to show that a certain amount of estrin is required to support the corpus luteum (Westman, 1934; W. M. Allen and Heckel, 1936; Robson, 1937). In view of the commonly-assumed suppressive action of the corpus luteum upon follicular growth, it may be that the production of estrogenic hormone by the corpus luteum is a means of providing against its own premature decadence through estrin-lack.

III. Estrogenic hormone in the placenta. The presence of large amounts of estrogenic hormone in the human placenta raises several very important questions, first of all the question as to whether this hormone is actually made in the placenta, or merely stored there.

In the human placenta the presence of estrogenic substance was early detected by the effect of placental extracts upon the castrate uterus and the immature uterus of the rabbit. After the introduction of the vaginal smear test quantitative studies were quickly made and the placenta found to yield an amount of hormone amounting to 400–700 rat units (Doisy, Ralls, Allen and Johnston, 1924).

Two placentas of the chimpanzee have been examined by Allen, Diddle, Burford and Elder (1936). In one case a whole placenta, weighing with the umbilical cord 290 grams, yielded 200 mouse units; in the other a 100 gram fragment yielded 200 mouse units.

With regard to the rhesus monkey, Allen, Maddux, and Kennedy (1931) reported qualitative evidence of the presence of estrogenic hormone in each of two full-term placentas.

The placentas of carnivores (cat and dog) were tentatively reported by Allen (1927) to contain no hormone.

About the estrogen content of the rodent placenta we have little information. Two workers in the reviewer's laboratory, Heckel and Suter, some years ago had negative tests from extracts of rabbits' placentas, indicating the presence of less than 1 rat unit in 4 grams of placental tissue (personal communication). Indirect evidence against the presence of active estrogen in the rat has been obtained by Selye and McKeown (1935). These writers have described a special effect of estrogenic substance, namely, production of an endometrial mole under certain experimental circumstances. This cannot be observed in rats having healthy placentas but deprived of their ovaries, i.e., estrogen is not made in the rat's placenta. Allen (1927) had negative results from a small amount of rat's placental tissue extracted directly.

In the case of the guinea pig, Courrier (1931) has pointed out a roundabout argument for the placental production of estrin. Herrick (1928) and Courrier have confirmed the general belief that in the guinea pig pregnancy may often continue after castration. In such animals the relaxation of the symphysis pubis, attributed by Fevold, Hisaw, and Meyer (1930) to a special hormone of the corpus luteum (relaxin), occurs as usual. Since, however, relaxin is supposed to require for its effect the previous action of estrogenic hormone, it follows that estrin has been available, presumably from the placenta. The argument is ingenious but involves too many uncertainties. The result of one thorough-going effort to extract estrin directly from the guinea pig's placenta would be much more valuable.

The domestic pig's placenta was reported negative, that of the mare positive, by Allen (1927) who also reported the presence of estrogenic substance in the chorionic cotyledons of the cow and sheep. Parkes and Bellerby (1927) obtained an average yield of 374 mouse units per kilogram from ten batches of cow's placental tissues, the extremes being 192 m.u. and 2123 m.u. From sheep's cotyledons (4 batches) the average yield was 161 m.u. per kilogram (extremes 116 and 308 m.u.). By way of comparison the same investigators obtained from the human placenta, as part of the same investigation, an average yield (36 placentas extracted separately) of 288 m.u. per kilogram (extremes 66 and 728 m.u.).

Parkes and Bellerby reason that the placenta is the site of storage, rather than production of estrogenic substance, because the hormone is also found in the amniotic fluid and the umbilical cord. Since these can scarcely be sites of production, the placenta is also by implication ruled out, in their opinion.

A possible means, not indeed of actually localizing origin of the placental estrogen in the tissue where it is found, but at least of ruling out the ovary, is afforded by those species in which total castration during pregnancy does not necessarily terminate gestation. Two such species seem well authenticated, besides the guinea pig already cited, namely, the cat (Gros, 1936) and the human (many reports). In our own species the occurrence and necessary removal of bilaterally cystic ovaries during pregnancy has given several opportunities for study of hormone output after castration. It was, in fact, this very condition which led Halban (1905) to formulate the hypothesis that the placenta is a site of hormone production.

Amati (1928) was the first to examine such a patient for the presence of hormone after castration, but unfortunately his results are not con-

clusive. The patient had both ovaries removed at about the 105th day of pregnancy. Three days after birth (which took place 165 days after operation) the blood serum was tested on immature guinea pigs and found to produce changes of the uterus and vagina attributed to hormone action. The test animals were not castrated and therefore the active substance may as well have been gonadotropic hormone (FSH) as estrin.

Five cases have however been studied by more specific tests.[2] Waldstein (1929) reports a patient bilaterally castrated at the 34th day of pregnancy. Delivery occurred normally at 270 days, with normal lactation. Post partum involution of the uterus was followed by general atrophy of the uterus accompanied by the usual symptoms of ovarian deprivation. During the last month of pregnancy the blood-serum contained about 1000 mouse units of estrogenic substance per liter and the urine 400 to 500 m.u. per liter. The placenta contained 300 m.u.

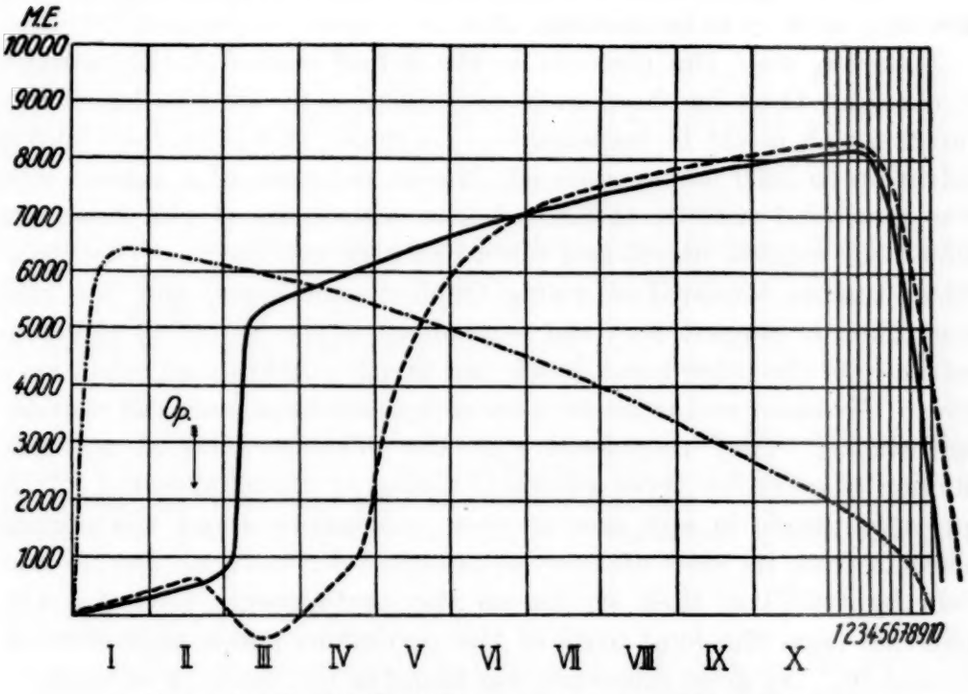
Szarka (1930) studied a case in which bilateral oöphorectomy was done in the 3rd month, with pregnancy to term, normal labor and lactation. Ten hours before birth the urine contained about 1000 to 2000 mouse units of estrin, an amount considerably higher than the non-pregnant woman excretes but lower than the usual excretion at the end of pregnancy. Blood from the umbilical cord and the retroplacental hematoma yielded estrin in amounts comparable to normal pregnancy. After parturition estrin in blood and urine dropped to very low figures.

The patient studied by von Probstner (1931) was castrated about the 50th day, and gave birth at about 280 days, with normal labor and lactation. Genital atrophy was observed four months after parturition. In this case the urinary estrogen content was assayed every 3 to 4 weeks throughout pregnancy, and that of the blood was tested near term and during the puerperium. As shown in the graph (fig. 2) after a preliminary drop the estrogen output returned to normal, reaching 8000 mouse units in the last months. It dropped to insignificant amounts within a few days after delivery. Von Probstner suggests that the preliminary drop after oöphorectomy means that in the third month the ovaries are still a significant source of estrogenic substance.

Allan and Dodds (1935) studied a patient castrated at about $3\frac{1}{2}$

[2] It must not be assumed because of these cases that the corpus luteum can be removed with impunity during pregnancy. Cf. Wilson (1937) who cites 5 cases of operative interference with the corpus luteum, all of which terminated in abortion. See also Asdell (1928).

months. Birth occurred at 10 months. Estrogen in the urine was assayed at 2000 mouse units per liter before operation. Fifty-four days after operation the yield was 1300 m.u. per liter. This was a drop of 40 per cent, but the output was of course still very much higher than that found in the non-pregnant subject. In a case of Saidl (1932) cited by Allan and Dodds, the output though lower before operation was not diminished by removal of the ovary.



——— Kurve des Ovarial-(Sexual)hormons bei einer Normalschwangeren.
– – – – – Kurve des Ovarial-(Sexual)hormons bei einer kastrierten Schwangeren.
–·–·–·– Kurve des Hypophysenvorderlappenhormons.

Fig. 2

Guldberg's case (1936) was actually studied in 1931. Double oöphorectomy occurred on the 113th day, as estimated from the history. Premature delivery took place on the 214th day. During the 100 days there were five examinations of estrogenic substance and gonadotropic hormones in blood and morning urine. The yield of estrin was high, reaching more than 17000 mouse units per liter in the morning urine and more than 400 mouse units per liter in the blood plasma. The placenta, which weighed 200 grams, contained more than 450 mouse units.

From such observations as these it is certain that an extra-ovarian source of the estrogen in placenta, blood, and urine during pregnancy must be postulated. That this source is the placenta itself remains the best hypothesis. Certain other possibilities, however, although they are not probabilities, remain to be excluded. The finding of estrogenic substances in muscle by Frank and colleagues, and the recently discovered presence in extracts of the adrenal gland, of substances resembling estrogenic hormone and progesterone (Callow and Parkes, loc. cit.) warn us to be cautious.

Assuming that the placenta is the actual source of the estrogen it contains, there has been some conjecture as to the histological elements which might be responsible. De Snoo (1928) reported finding estrin up to 1000 mouse units per liter in the urine of a patient who was moribund with metastatic chorionepithelioma of the tube, for which the ovaries, uterus and tubes had been extirpated. The metastatic tumors consisted of young trophoblastic tissue, and the case was taken to suggest that the trophoblast is the source of placental estrin. On the other hand Smith and Smith (1935) found estrin very low in the blood and urine in cases of hydatidiform mole and chorionepithelioma. They considered that the evidence hints at non-production of estrin by these abnormal tissues of placental origin. It is probably unsafe in any case to draw conclusions about the normal placenta from its most extreme pathological derivatives. Parkes and Bellerby (1927) in their studies on the cow placenta separated the maternal from the fetal parts of the cotyledons and extracted them separately. No great difference was found in the yields of estrogen.

GENERAL CONCLUSIONS

1. The estrogenic hormone found in the ovary and in the blood and urine of the non-pregnant female is probably produced in the ovary. The most probable site of production in the ovary is the theca interna of follicles of all sizes.

2. The estrogenic hormone found in the placenta and in the blood and urine of the pregnant female of certain species, including the human, is produced by an extra-ovarian source, almost certainly the placenta.

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AUTOLYSIS AND ATROPHY

H. C. BRADLEY

Laboratory of Physiological Chemistry, University of Wisconsin

In the fifteen years which have elapsed since this field was reviewed (1) a very extensive literature has accumulated, and some notable advances have been made. The scope of the *in vitro* experimentation has been greatly extended; the ease and accuracy of various measurements, such as pH, has increased. Interpretations offered in 1922 require careful reappraisal and revision now.

Because the various abstract journals, annual reviews, and special monographs now available cover the literature so thoroughly, it does not seem profitable to cite the entire literature pertinent to this subject. We shall, however, attempt to give as consistent an account of the autolytic mechanism as can be formulated at this time and to relate it again to the phenomena of atrophy and hypertrophy. Although the studies of autolysis are all made upon tissues post mortem, there is justification in attempting to project such reactions back into the physiology of the living cell. And while it is true, as has been frequently pointed out (2, 3) that mechanisms appearing post mortem may not be operative in the living cell, nevertheless, until some other mechanism is discovered with which to relate the atrophic shrinkages of surviving tissue cells, and their return to normal mass again in hypertrophy, we are justified in assuming the autolytic mechanism as the probable one involved.

MATERIALS. The great mass of data upon autolysis has come from mammalian tissue—chiefly liver, kidney, and spleen. Practically all other mammalian structures have, however, been studied sufficiently to indicate that the mechanism is universally present. The only exceptions, so far as we know, are the connective tissue group, white fibrous, yellow elastic tissue, cartilage, and bone. It is possible that even in this group autolysis goes on, but at a rate too slight to be detected by the usual methods. For practical purposes, the connective tissue group is devoid of autolytic enzymes, and is resistant to digestion. Once laid down, it remains *in situ* unless it becomes the locus of phagocytic invasion.

Not only is the characteristic mechanism present in all mammalian

tissues, but it has been found in lower vertebrate forms so far as these have been investigated, and in invertebrates (4, 5, 6, 7). The same mechanism appears to exist in the embryonic tissues studied (8, 9, 10, 11, 12). The phenomenon of autolysis, therefore, gives evidence of being as widely present in animal cells, as is respiration and metabolism itself. This alone would justify the assumption that it is of physiological importance—until the contrary becomes apparent.

The autolytic phenomena. Since the picture of the autolytic mechanism is so closely alike from one tissue to another—where it is not overlaid by some more active proteolytic mechanism such as exists in the pancreas and gastric mucosa—we shall give a brief description of it as it occurs in the mammalian liver. Quantitatively this description will differ from kidney, spleen, muscle, or brain. But in its broader features the mechanism is qualitatively the same, and the differences are attributable quite as much to the differences in the properties of the proteins of the tissues as to qualitative differences in the enzyme systems.

pH. If liver is finely comminuted immediately after death and dispersed in water, the first recognizable change develops in the H ion concentration (13). We may assume the pH of liver cells is not far from that of blood—the Donnan equilibrium would indicate about 7.2, and the known pH of the corpuscles confirms this assumption. The work of Chambers indicates that pH 6.9 may be a rather constant figure, found in a great variety of cells (14). This is an average pH value, and does not preclude areas of considerably higher or lower H ion concentration within the cell. This average pH changes rapidly after death. Within four minutes the liver tissue has a pH of 6.8 or less, and within an hour it may reach 6.2. Thereafter there is a slow drift of the reaction back toward neutrality for a few days and then again a slow movement in the acid direction. These later changes seldom exceed 0.2± on the pH scale. They are referable to the cleavage products that are being produced in autolysis and the changes in the buffering efficiency of the tissue brei.

If the liver brei is set at various pH levels by the addition of acids or alkalies, the reaction of the digest undergoes characteristic shifts which are indicated in the following chart. Note particularly the very rapid reduction of alkalinity that occurs in the tissue starting at pH 9+, in which autolysis is minimal or zero.

The acids produced in a control digest of the liver are H_3PO_4, carbonic, fatty acids, and lactic acid in the order of their amounts. In the breis made alkaline, so that little or no proteolysis occurs, the production

of phosphoric and fatty acids may, nevertheless, increase as much as 300 per cent over the initial concentration found, and the phosphoric acid is ten times as important as is the lactic acid. In a more detailed study of the phosphoric acid liberated in autolyzing liver, Sevringhaus (15, 16) showed that in the earliest samples obtainable post mortem, liver P was not far from that of normal blood—12 to 20 mgm. per 100 grams tissue. There is rapid rise for several hours indicating an enzymic liberation, which then continues more slowly for the duration of the autolysis. In six days the inorganic phosphate of the liver may reach 300 mgm./100 grams tissue. It is interesting to contrast the liver with muscle of the same animal. The production of phosphoric acid post mortem in the muscle is much more rapid than in liver during

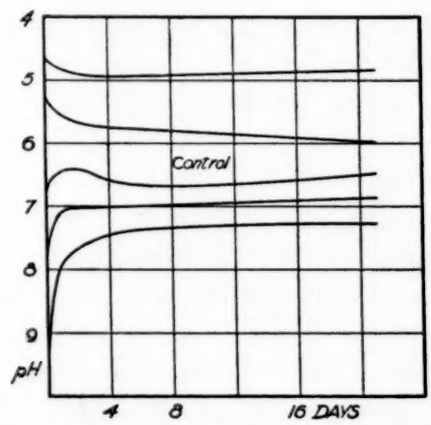


Fig. 1. pH changes autolyzing liver brei

the first hour, but is nearly complete at about 80 mgm./100 grams tissue. The increases after the second hour are small.

Proteolysis. When a liver brei, prepared as rapidly as possible after death, is sampled and precipitated by trichloracetic acid, the filtrate is found to contain: a small but rather constant amount of non-precipitable N; a low but definite figure for amino acids by the formol titration; a faint color with the Folin phenol reagent (17), indicating reactive tyrosine-peptides, tyrosine, or other protein decomposition products which reduce this reagent; a small amount of amino N as estimated by the Van Slyke nitrous acid techniques (18). These figures are rather characteristic of the tissue being studied. They constitute the base line from which the evidence of proteolysis must be reckoned. In the liver, about 10 to 20 per cent of the total N eventually solubilized in autolysis under the most favorable conditions is already present in the tissue in

non-precipitable form. About the same proportion of the amino acid which becomes titratable during such an autolysis is already present. About 4 to 7 per cent of the total tyrosine is present in reactive form. The constancy of these figures indicates an equilibrium which exists between these small molecular fragments and the tissue proteins on the one hand, and the blood and lymph on the other.

Digestion begins at once if measured by the solubilizing of protein N, or by the unmasking of reactive tyrosine. Formol titration and amino N are less effective in determining early cleavages, and as the digestion continues give figures which represent the composite cleavages by both the proteinase and the peptidases present.

Methods. The increase of non-precipitable N, or the decrease of the precipitable N constitutes the most direct evidence we have of primary cleavage, and is the oldest method used in the study of autolysis.

Colorimetric determination of tyrosine offers a convenient technique for determining cleavage in autolysis, and has been found to correlate most closely with the primary cleavage by proteinase (19, 20, 21). The recent application of this technique by Anson (22, 23) to pepsin, trypsin, papain, and cathepsin studies, on a standard hemoglobin substrate, gives a particularly clear definition of the primary cleavage.

The formol titration of amino acids is so easily made that it has been widely adopted, and a great deal of the data in the literature is in terms of the amino acid titre. Unfortunately this does not serve to clearly differentiate primary from secondary cleavages. Where the digestion time is short, the formol titration figures approximate proteinase action. On the other hand, the formol figures on a short digest are so small and the errors relatively so great that digests are usually sampled at the end of the first twenty-four hours. By this time the figures are certainly a composite, and represent all types of cleavage.

The original formol titration of amino acids by Sørensen (24) has been modified in a number of ways to increase its accuracy or to give better agreement with the Van Slyke methods for amino N determination (18). Thymol Blue has been substituted for phenolphthalein, and Northrop advocates the use of two indicators: Neutral Red for the first neutralization; Thymol Blue for the titration of the amino acids (25). The effect of this is to increase the spread of the titration, and to give a sharper end point.

In some of the European laboratories, strong alcohol is used instead of formaldehyde. This suppresses the basic properties of the amino acid and allows titration of the carboxyl groups (26). It also permits some differentiation between the peptide carboxyls and those of the

amino acids, since peptide carboxyls may be titrated in 50 per cent alcohol, while 90 per cent or above is required for titrating the amino acids.

Latent period. While a latent period of about four hours was described by Lane-Claypon and Schryver (27), it has not been confirmed (28). If non-coagulable N is accepted as adequate evidence of proteolysis, there is clear indication that cleavage is under way in the control liver brei within two hours. If the H ion level is raised to pH 4–5, digestion is evident in 15 minutes. If alkali is added to a liver brei, there may be no evidence of digestion in 24 hours. We conclude, therefore, that there is no latent period where there is an abundance of substrate, and digestion is rapid. Where the conditions for autolysis are adverse, digestion is so slow that there appears to be a latent period merely because of the limitations of the methods used to detect cleavage.

Effect of reaction. It has long been known that acidity increases autolysis (29). Our own studies have led us to conclude that the reaction of the tissue brei determines the speed and amount of digestion by fixing the amount of available substrate for the tissue enzymes to act upon (30). This conclusion is not in any way at variance with the phenomenon of sulfhydryl activation of cathepsin, to be discussed in more detail later. It does seem to indicate that there is active proteinase present in the liver immediately post mortem. If Waldschmidt-Leitz is correct in his statement that an activator is produced and increased by autolysis, there must obviously be some autolytic activity already present to produce it (31, 32). Our own digestion curves have never shown the inflection at the start which should be found in an autocatalytic reaction. They appear to rise most steeply at the beginning of the reaction, and flatten out later as the digestion approaches an equilibrium. Nor does the final equilibrium depend upon the destruction of the enzyme. After autolysis has proceeded three weeks, and the reaction is at a standstill, the addition of digestible protein leads to a resumption of the proteolysis. The enzyme, therefore, has not disappeared, and the final plateau of the digestion curve represents the completed reaction where all protein which is accessible has been digested. The reason digestion attains a higher level at pH 4 than it does at pH 6 is because at pH 4 there is considerably more protein available to digestion. The relation between acid added and the amount of cleavage products formed in a liver is almost a stoichiometric one. A better correlation, however, appears to exist between the pH of the brei, and the amount of cleavage products developed.

Our original assumption was that the tissue proteinase can attack

only the acid-protein salt, or the protein cation (19). Waldschmidt-Leitz and others believe it is the free, or iso-electric protein which cathepsin attacks (31, 33), and our own results may be interpreted to support this view. In figure 2-A is shown the progress of digestion in autolyzing liver started at various pH levels (34).

Digestion of foreign proteins. Many foreign proteins have been used in the study of autolysis, with a view to discovering specific properties of the enzymes present. Some, like peptone and gelatin, are digested in both slightly acid and slightly alkaline breis. Casein, fibrin, albumins and globulins, clupein, hemoglobin, and edestin are all found to digest well in acidified tissue breis, though the degree of acidity is important in determining individual behavior. Gelatin, which has been used most as a substratum, is unsatisfactory in the investigation of tissue proteinase since it is not a typical native protein, is probably

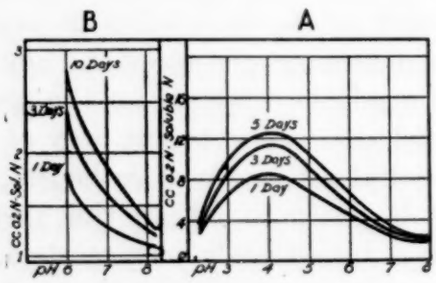


Fig. 2-B and 2-A. The autolysis of liver at various H ion levels

digested by other enzymes, and is not digested by purified cathepsin itself (23). Much of the published work, therefore, on tissue proteinase will require reinvestigation before it can be given unqualified acceptance, because the substrate was gelatin.

With a limited group of rather resistant proteins, the iso-electric point has been suggested as a determining factor in digestibility (20, 35). Hemoglobin, with an iso-electric point of pH 6.8 is slightly digested by liver enzymes between pH 7 and 5. At higher acidity it is extensively digested, with a maximum in the neighborhood of 4. Serum itself, egg white, and the purified albumins and globulins from these sources, are all found to inhibit the autolysis of liver at H ion concentrations below pH 5. Above this critical point these proteins become available substrata for the proteinase of liver. Edestin shows no inhibition and is slightly digested at pH 6.5. It is increasingly digested as the H ion rises, with a maximum availability at pH 4.5. Its critical point is in

the neighborhood of pH 7.0. The relation of the H ion level with the digestibility of these foreign proteins is illustrated in figure 3.

The correlation between the digestibility of these proteins and their iso-electric points is illustrated in table 1.

The phenomenon of inhibition of the autolysis of liver proteins by such resistant foreign proteins as ovalbumin and serum globulin has been interpreted as indicating a partial fixation of the proteinase by albumin, at a pH range where the latter is not fragile. The enzyme

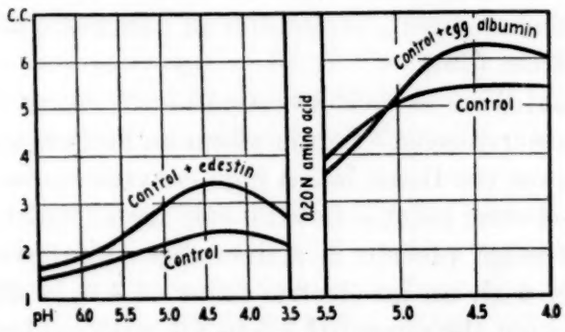


Fig. 3. The effect of pH on digestibility of foreign proteins in autolyzing liver brei.

TABLE 1

Iso-electric points and availability in a liver digest

PROTEIN	ISO-ELECTRIC POINT	CRITICAL POINT
	pH	*pH*
Egg albumin	4.8	5.0
Egg globulin	5.2	5.3
Egg white		5.5–5.3
Serum		4.6
Serum globulin	4.4	4.8
Serum albumin	4.7	5.0
Edestin	6.9	7.0 (?)

appears to be strongly adsorbed, and thus removed from the field of activity. When the H ion concentration is raised to the critical point, the enzyme is less strongly bound by the albumin; therefore, it neither digests nor inhibits the digestion of the available liver proteins. When the H ion of the brei is still further elevated, the albumin becomes increasingly fragile, and so contributes to the total available substratum of the mixture. At about pH 4, it is completely digested. The gradual shift from active inhibition to complete digestibility is clearly related to

the H ion concentration present, and appears to correlate with the change in physical and chemical properties of the protein as it passes from base- to acid-protein salt through the iso-electric point.

The inhibitory effect of egg white and serum proteins occurs whether the untreated serum or egg white is added, or the individual proteins purified by repeated recrystallization or precipitation. It seems probable, therefore, that this type of inhibition is produced by the protein itself rather than by some other inhibiting agent present. The degree of inhibition is determined by the amount of the resistant protein added. Thus in a prolonged digest, ovalbumin in increasing amount produces increased inhibition (35).

Kleinmann and Werr in their investigation of cancer enzymes in 1932 report similar observations. Serum albumin, and organ albumins were not digested unless the tissue had a high enzyme content (36).

That the iso-electric point is not the only factor which determines the behavior of a foreign protein in a liver digest, however, is shown by casein. Casein, with an iso-electric point of 4.6, is digested by liver enzymes throughout the range pH 7.5 to 3.0, with evidence of one maximum at about pH 4.0, and a second about 6.0. In their studies of the Jensen rat sarcoma, Voegtlin and Maver found the digestion of fibrin also presented two maxima, one between pH 4 and 5, and another at pH 7 (37). Both casein and fibrin are known to be easily digestible proteins, and are very likely fragmented by other enzymes present with the tissue proteinase. Edestin, while more easily digested than serum or egg albumins and globulins, is more resistant to cleavage than casein. It does not inhibit the cleavage of the liver proteins by the tissue proteinase, however, as do albumin and globulin.

In addition to the iso-electric point, as a determinant in the behavior of added protein, therefore, we must also include the less well defined property of the individual proteins, which may be called fragility. In general, we are familiar with the fact that casein, fibrin, and denatured hemoglobin are readily digested in the alimentary tract; the raw albumins and globulin are more resistant; collagen, elastin, and keratin may be completely resistant.

In the case of liver, there is very little digestion of the tissue proteins possible at pH 7.5. If the proteinase is active at this pH—an unsettled point—there is no substratum available and the enzyme is probably fixed by adsorption to base-protein salts which are resistant at this pH. Thus, under normal cell conditions the system is stable, and hydrolysis cannot occur. As the H ion concentration rises, the liver proteins

become increasingly fragile and the enzyme less firmly adsorbed. When it combines with proteins with sufficient open —COOH groups to be fragile, fragmentation occurs. At pH 6.8 about 50 per cent of the total liver proteins (exclusive of the wholly resistant sclero-protein group) is fragmented in a prolonged digestion. At pH 4, 98 per cent of the liver proteins are digested. This, we believe, is important in attempting to relate the autolytic mechanism to the phenomena of physiological or pathological atrophies. Within the normal range of cell reaction it is possible to predicate as much as a 50 per cent removal of cell proteins during a prolonged digestion period. Most physiological atrophies are relatively gradual and prolonged. From this point of view it is more important to determine what happens within the range pH 6.8 to 7.5, than it is to determine the behavior of tissues at the optimum pH of 4.5.

In figure 2-B is shown the relation of the H ion concentration to proteolysis within the physiological range of reaction variation in a tissue. The curves represent the changes in soluble N over a ten-day period. The pH levels, it will be recalled, are never static. The more alkaline breis grow less alkaline even when heavily buffered, and the use of concentrated buffers is not entirely satisfactory since they themselves tend to inhibit autolysis. The curves are drawn for the initial pH values, but by the end of the ten-day digestion period they have shifted considerably in the acid direction. This shift is the more pronounced, the more alkaline the brei, and undoubtedly accounts for some of the cleavage found in the more alkaline units of the series.

Muscle autolysis and atrophy. The autolysis of muscle tissue does not offer any striking qualitative differences from that of liver and other glandular organs, but is sufficiently different in its quantitative aspects to warrant some detailed consideration.

While the mechanism of autolysis is the same as that found in liver (38), the muscle proteins themselves are predominantly resistant to catheptic action *in vitro*. Thus while liver contains about 15 per cent non-digestible scleroproteins after prolonged autolysis, and the remaining 85 per cent is completely digested, muscle contains 70 per cent of its protein in a form resistant to autolysis under optimum conditions. The shreds of striated muscle tissue in a typical autolysis retain indefinitely their characteristic striations. Muscle structure gives the impression of great permanence in the digestion *in vitro*, which is not altogether in harmony with muscular atrophy. It is well known that an inactivated or denervated muscle may lose a very large fraction of its mass through

disuse. The behavior of the muscle *in situ*, therefore, poses an interesting problem when one tries to relate muscular atrophy to muscle autolysis.

When a muscle is examined immediately after death, it is found to develop the same type of pH changes which were found in liver. The muscle, however, may attain much higher H ion concentrations post mortem—as, for example, pH 5.7. The same slow shifts in the H ion develop during autolysis as are found in liver. The liberation of phosphoric acid is more sudden in muscle than in liver, but the final amount is much less. The process is complete by the end of two hours, post mortem.

If a muscle brei is acidified, the optimum point for autolysis lies in the range pH 4–5. In such digests, the protein solubilized is about 30 per cent of the total muscle protein. The active red muscle of the chicken's leg autolyzes considerably more extensively than the inactive white muscles of the breast. There is more potential protein substratum present—which may be merely the additional digestible muscle-hemoglobin present in the red muscle. The red muscles of the rabbit, beef, and dog, autolyze better than the white chicken muscle. Cardiac muscle autolyzes to about the same extent as active skeletal muscle, while the paler smooth muscle of the mammal autolyzes to the same extent as white muscle of the fowl. Frog muscle also autolyzes like active mammalian muscle.

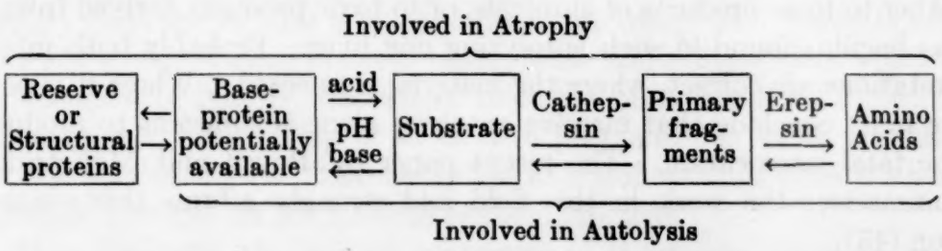
In the lower vertebrates and invertebrates there is wide variation found. From our limited data there is some evidence of a correlation between activity and the extent of autolysis. The most extensively autolyzing fish muscle we have studied is that of the very active mackerel. Seventy per cent of its muscle may be solubilized in prolonged autolysis. In contrast to the mackerel, the muscle of perch, carp, scup, and the sharks are characterized by very small autolytic changes.

Observations on the autolysis of salmon muscle at the beginning of their migration and at the end would be of particular interest. Greene (39) has shown that the muscle protein is reduced 30 per cent. During the migration the salmon eats nothing, and at the same time the gonads hypertrophy and mature. Here, then, is a physiological atrophy in which 30 per cent of the muscle proteins are mobilized for fuel and for growth of other organs. In the autolysis of salmon muscle, obtained on the market, not more than 18 per cent of the protein was found to be solubilized in a prolonged digest.[1] The discrepancy between this figure

[1] Unpublished data.

and the 30 per cent protein loss in migrating salmon cannot as yet be explained.

In their study of the atrophy of denervated mammalian muscle, Chen, Meek, and Bradley (40) paralyzed one hind leg in a series of 19 rabbits. The inactive leg was protected against injury and infection by a padded leather boot. The progressive changes in the atrophic leg muscles were then compared with the normal leg muscles as control. The animals were sacrificed in groups of three each week, over a period of ten weeks. At the end of this period the denervated muscles were reduced to 50 per cent of the mass of normal controls. The curve of weight loss is logarithmic and tends to approach an equilibrium in ten weeks much as does an autolytic curve. There was practically no difference in the water content between normal and atrophic muscles. There was no difference in the pH values immediately post mortem and after 24 hours' autolysis. The buffer value against acids and alkalies was not altered significantly by atrophic changes. The denervated muscles had already lost 50 per cent of their total protein mass, and yet the autolysis of the denervated muscles was more extensive than the normals, in control, acid and alkaline breis. The initial tyrosine values in the atrophic muscles were always significantly higher than in the normal, which indicates a slight accumulation of protein fragments. There was, however, no difference in the amino acid titres of the initial samples. At the end of autolysis there was more reactive tyrosine in the atrophic than in the normal breis. There is a striking constancy in the autolysis figures in muscles which had atrophied from one to ten weeks. This suggests an equilibrium between the mass of resistant proteins, unavailable in the tissue digests, and the mass of potentially available proteins present. We have attempted to schematize the relations which this study suggests in the following diagram:



The mechanism by which the unavailable structural proteins of muscle are gradually converted into available substratum has not been discovered. There was somewhat more digestion of the atrophic tissue in alkaline media than the normal, as measured by both formol titration

and tyrosine, though as usual these figures are relatively small. Almost identical differences were found between normal and atrophic breis digesting at pH 6. It is possible that there is leucocytic tryptase present in very small amount, though careful examination of the histological sections failed to reveal any evidence of infiltration by leucocytes. Nor is it clear how extra cellular tryptase could digest proteins within the living cells.

Clinical autolysis. The slow removal of tissue proteins by autolysis under physiological conditions is accompanied by no evidence of intoxication. The breakdown of tissue proteins into primary cleavage products is correlated with the further disintegration of these fragments into the final products—small molecule peptides and amino acids. On the other hand, massive autolysis may result in the escape of intermediate fragments into the blood stream, with toxic results. In the original studies by Jacoby (41), on acute atrophies of the liver, as in phosphorus poisoning, it may be inferred that part of the symptoms of the animal were due to products of autolysis, toxic in character. Mann (42) found that animals survived complete removal of the liver better than they did the operations in which some traumatized liver masses were left in the abdominal cavity. The problem of defining the cause of death in animals supporting a mass of autolyzing tissue has recently been intensively studied from both the experimental and the clinical side. The increase of traumatic injuries to liver and other abdominal organs due to the automobile, and the frequent instances of a fatal syndrome known as "liver death," has intensified the interest of clinicians and pathologists in this field.

Thus far, the results of numerous investigations have not entirely settled the matter. In many animal experiments, the fatal outcome of leaving a piece of liver in the abdominal cavity has been attributed either to toxic products of autolysis, or to toxic products derived from a gas bacillus found in such autolyzing dog liver. Probably both interpretations are correct, where the material is infected. Where it is not, we must conclude that massive autolysis alone is sufficient to produce the fatal intoxication. The recent paper by Boyce and McFetridge summarizes the work in this field and strongly affirms this conclusion (43).

Pathological changes in the lens, vitreous humor, and retina have been correlated with the autolytic mechanism experimentally studied in these structures by A. C. Krause (44).

The autolytic enzymes. Jacoby in 1900 (41) was the first to attempt a

separation and characterization of liver enzymes. By fractional precipitation of an autolyzing liver brei he obtained an enzyme, or enzyme group, having the properties of erepsin. In 1904 Hedin described two enzyme entities obtained from the spleen, which he designated alpha and beta protease. Alpha protease acts in alkaline media, and is probably referable to the leucocytes present in the spleen (45). Beta protease is active only in acid media and is responsible for the primary cleavages of protein *post mortem*.

In 1905 Vernon (46) described the widespread distribution of erepsin-like enzymes in tissues. Excluding intestinal mucosa, the ereptic activity is found in the order: kidney, spleen, pancreas, liver, cardiac muscle, and skeletal muscle. This study requires repetition with more exact and discriminating modern technique.

In 1917–18 Dernby (47) concluded that the enzyme complex involved in autolysis consists of pepsin, trypsin, and erepsin. Dernby's own result, however, indicated important differences between these enzymes of the digestive tract, and the proteinase of tissues. Subsequent work has emphasized the difference and has indicated that a single proteinase occurs in tissues, closely associated with an enzyme group resembling erepsin of intestinal juice (19). The primary protease, or proteinase, is active between pH 7 and 3, with an optimum at pH 4–5. It is responsible for the initial cleavage of available tissue proteins or added substrata such as casein or albumin. The erepsin is concerned with the secondary cleavages of the primary fragments, and is active over a wide range, with an optimum about pH 7–8. It is not uncommon to find massive separations of tyrosine crystals in liver digests set at about pH 4–5, which indicate clearly that cleavage goes on to the final products at such H ion levels. The primary cleavage of the available substratum is complete in a prolonged digest. The secondary cleavage *in vitro* is apparently never complete. There is evidence of an equilibrium between amino acids and polypeptides which is independent of the pH, and whose value has been calculated to be the constant 0.56 (35). This is an *in vitro* phenomenon, where cleavage products accumulate. It probably has no significance in a tissue where diffusion provides for the removal of these products.

Thus, by 1929, the general properties of the tissue proteases were fairly well defined as they could be made out from studies of the tissue brei. Willstätter and Baman (48) at that time were able to separate from the pepsin of gastric mucosa a proteinase acting in weak acidity on such proteins as gelatin, clupein, and egg albumin. For it they

proposed the name "Kathepsin," from the Greek, "to digest." Closely associated with it was erepsin, identified by its ability to cleave d-l leucylglycine at pH 7.5.–8.6. The name kathepsin, or cathepsin, has been generally adopted since that time, as distinctive for the proteinase of animal tissues. Intensive studies of its distribution followed immediately. Its specific characteristics have been amplified, together with methods of separating, purifying, and measuring its activity.

Thus Waldschmidt-Leitz (31, 32) found cathepsin in rat tumors and considered it of great significance. Kleinmann and Werr extracted cathepsin from human tumor tissue, and compared activity with that of normal tissue. They found neither qualitative nor quantitative differences of significance. The amount of cathepsin was correlated with the actual cellular content of the tissue (36). Several other investigators confirm the presence of cathepsin in tumor tissue, but differ in their interpretations of its significance (49, 50). There is no doubt that the mechanism is operative in softening any necrotic areas of tumors.

Cathepsin, active trypsin, and peptidases have been found in the polymorphonuclear leucocytes, while lymphocytes and mono-nucleated cells contain cathepsin and peptidases (51). Such free cells are easily obtained in quantity and are uncontaminated by other tissue factors. Some species differences are found in the enzyme patterns of the leucocytes (52, 53).

Complete protease systems have been identified in spleen and liver, consisting of cathepsin, carboxypolypeptidases, aminopolypeptidase, and dipeptidase (54). Recently Anson has obtained carboxypolypeptidase in crystal form, which digests chloracetyl-tyrosine and other synthetic substrata (55), which are useful, therefore, in detecting and measuring its activity. In the salivary glands cathepsin and some active trypsin were found (56), but no pepsin. The trypsin is probably derived from leucocytes. In cell-free saliva the only enzyme demonstrated was amylase. Embryonic tissues contain cathepsin (9, 12), which seems to be related in amount to the rapidity of tissue synthesis going on.

Not all of the cathepsin in tissues exists in the free state (57). A fraction of it is firmly bound to the insoluble proteins of the tissue and so is not extracted. The bound enzyme Willstätter called "desmocathepsin"; the readily extractable enzyme, "lyo-cathepsin." The desmo- form may be liberated by weak acid, or by allowing moderate autolysis to take place in aqueous glycerol. The two forms are then

found to be identical. This appears to us to correspond to the fixation of the enzyme by stable base-proteins which in this case are also insoluble, and which we have previously discussed.

The phenomenon of activation. In 1928 Grassmann and Dyckerhoff (58) succeeded in separating the proteinase of yeast from its associated polypeptidase, in relatively pure form. The polypeptidase was inhibited by HCN and H_2S, while the proteinase was rendered more active. If the purified inactive proteinase was allowed to stand, a natural activator developed, whose effect was analogous to that of HCN and H_2S. The freshly purified enzyme would split peptone, but not the native proteins. On standing, or in the presence of HCN and H_2S, it readily digested casein, fibrin, gelatin, and histone.

Waldschmidt-Leitz et al. (31) made similar observations on spleen cathepsin the following year. The natural activator, which they later called Zookinase, they believed was produced by slight autolysis. Its effect was also to enhance the range of the enzyme. Like the yeast proteinase, the inactive spleen cathepsin digested certain protein cleavage products, but was without action on native proteins. In the presence of the activator, or HCN and H_2S, the enzyme was found to split egg albumin, gelatin, and protamine. The activator could be removed by adsorption on kaolin from the tissue extract, and was later crystallized and identified as reduced glutathione (G—SH) (59, 60). Its mode of action was not clarified, although the removal of an inhibiting agent present in the tissue was a current suggestion (61, 62, 63). The inhibitor might be a heavy metal ion, with which the activator forms a complex. On the other hand, Grassmann et al. (64) found that pyrophosphate, which also forms complexes with the heavy metals, has no activating effect. Pancreatic trypsin is inhibited by HCN, H_2S, cysteine, and pyrophosphate. Cysteine and enterokinase thus appear antagonistic in their action on trypsin. Cystine, however, can activate trypsin and will replace the enterokinase. Thus cysteine and cystine are seen to be antagonistic, and we have two natural proteinase systems in which these two compounds exercise opposite and antagonistic effects.

The interest which these papers, published between 1929 and 1931, has aroused is illustrated by the very large number of contributions to the subject which have subsequently appeared. For a more detailed review of the field, the reader is referred to the recent monograph by Pozzi (65). The list of activators has grown to large proportions. Sulfhydryl compounds, however, appear to be the factors of immediate

importance, and among these the beta sulfhydryls are apparently specific (66). Other reducing compounds such as ascorbic acid, especially in the presence of iron, reductone and reductinic acid (67, 68), SO_2 (69, 70, 71, 72), the succinate dehydrogenase, and ultra violet radiation, all appear to be effective activators by increasing or preserving −SH groups in the system. This led to the idea that chemotherapeutic agents which are known to affect −SH—such as organic arsenicals—may also influence the tissue catheptic system, and this has been demonstrated (73).

In general, then, we may conclude that the presence of such beta sulfhydryl compounds as reduced glutathione, cysteine, and protein −SH provide an activating mechanism for the catheptic system. Substances which inactivate or inhibit cathepsin are likewise those which abolish the −SH groups or oxidize them to −SS−. Cystine, SS−glutathione, produce no activation. Iodoacetic acid inhibits, so long as it is present (74). Cu inhibits and catalyzes the oxidation of −SH to −SS (75). Peroxide of hydrogen, alloxan, acetaldehyde, the aerobic dehydrogenase-hypoxanthine-peroxidase system, and exposure to high O_2 tensions were later shown to inhibit proteolysis by cathepsin (71), and to abolish −SH.

The broader implications of the activation phenomena had in the meantime been suggested by Waldschmidt-Leitz and co-workers (76, 77) in 1933. The conception of a general relationship between the state of the oxido-reductive systems of the cell, and the direction of its enzyme-catalyzed reactions was beginning to take form. It was found that one group of enzymes was activated by −SH, with or without the presence of Fe, while another group was inhibited. Thus papain, cathepsin, arginase, and alcoholic fermentation are activated. Phosphatase, catalase, and glyoxalase are inhibited. Iodoacetic acid reverses the effect of −SH in both groups. This attempt to see the enzymic processes of living cells in relation to the simultaneous influence of other enzymes and factors present at the same time and place, while purely exploratory, is of considerable interest, more particularly since it involves the concept of hydrolysis and synthesis regulated through the mediation of the −SH: −SS− balance. This is itself determined by the oxido-reductive systems of the cell. Thus a high blood supply, providing relatively high O_2 tension and low −SH values in a tissue adequately buffered to near neutrality, would appear to be the conditions making for tissue stability. The reverse conditions are known to lead to autolytic disintegration. The important new factors in this concept are those provided by sulfhydryl activation and the oxido-reductive system.

In concluding this portion of our discussion, it would be incorrect to leave the impression that the data are all harmonious. While the majority of investigations report activation by sulfhydryl, a very considerable number fail to detect the phenomenon. Some investigators, like Kleinmann and his co-workers, find cathepsin can be activated against gelatin, but not against casein, edestin, serum albumin, etc. (36). The crude enzyme is activated by HCN and H_2S, while the purified preparation can only be activated by H_2S. In a recent paper Anson (23) reports the preparation of a highly active cathepsin, containing no carboxypeptidase. This preparation, measured by ten minutes' cleavage of a standard hemoglobin substrate, is found to have a potency comparable to that of crystalline pepsin or trypsin. It does not digest peptides; it shows no increase of activity in the presence of cysteine; it does not digest gelatin. Its properties are such as to suggest that most of the previous enzyme preparations studied and described are impure. Since carboxypeptidase digests gelatin, is activated by sulphydryl, and is apparently very closely associated with cathepsin, the suspicion that it has been responsible for some of the results obtained is difficult to avoid. Bergmann finds evidence of two catheptic enzymes in liver (78). Another point of possible divergence lies in the presence or absence of hypothetical inhibitory compounds. Since practically all of the European work has been with extracts, there is room for much divergence in the preparation of the extract and its purification by adsorption or precipitation. If, for example, the cathepsin of Waldschmidt-Leitz contains an inhibitory substance which $-SH$ can remove, while Anson's highly active enzyme is free from it, such contradictions in the data may be harmonized.

Anson suggests that since cysteine delays the destruction of cathepsin in acid media, it simulates activation of the enzyme. It is clear that various cathepsin preparations obtained from the same tissue may differ in their properties. In our judgment, and pending clarification of points of difference, it appears that sulfhydryl activation occurs both in the tissue brei, as shown by Voegtlin and Maver, and in most of the tissue extracts which have been studied. The activation may be due to the production of $-SH$ compounds in the enzyme itself, or it may involve the production of $-SH$ in the substrate protein. It is interesting that in Kleinmann's numerous observations, gelatin is the only protein which requires H_2S for its digestion when cathepsin is sufficiently pure. The other proteins used were digested without sulfhydryl treatment. Gelatin is the only one of the group which contains no cystine. It is also suggestive that keratin, while rich in cystine is not

digested by any of the common proteinases, until its −SS− is reduced to −SH by alkaline thiol compounds, as has been shown by Goddard and Michaelis (79). It is possible that the effect of reducing reactions, and specifically −SH compounds, is to increase the fragility of the substrate proteins.

It should also be pointed out that studies of tissue breis are not directly comparable with studies of tissue extracts, or isolated enzymes. When the enzyme is finally obtained pure, it is acting under essentially artificial conditions. On the other hand, the study of a tissue brei aims to maintain the complications present in the tissue, in the hope of throwing light upon the still more complicated situations of living material. Nevertheless, the two methods are found to harmonize surprisingly well. Thus the extension of autolysis in a brei at fixed pH levels by −SH, as shown by Voegtlin, is in harmony with the activation of proteolysis as obtained with partially purified cathepsin extracts. The determination of the optimum pH for autolysis of a brei corresponds closely with the optimum found for cathepsin itself.

The behavior of the autolytic proteases found in liver and spleen may be tentatively summarized as follows:

Cathepsin. A proteinase acting on iso-electric proteins at an optimum pH of 4 +. It is activated by −SH (both affirmed and denied) and by reducing agents which convert −SS− into −SH. It is inhibited by O_2 and oxidizing agents which convert −SH into −SS−, such as high O_2 tension, H_2O_2, and Cu ions. It produces limited primary cleavages of the native protein molecule only.

Carboxypolypeptidase. A peptidase acting upon polypeptides and splitting off the amino acid having a free −COOH group, at an optimum pH of 4 +. Terminal tyrosine, tryptophane, and cystine are particularly favorable points of attack. For characterization, the pentapeptide, leucyl-triglycyl-glycine, chloracetyl-tyrosine, and a formaldehyde-treated peptic digest are useful and susceptible to cleavage by no other known enzyme.

(*Note:* The crystalline carboxypolypeptidase described recently by Anson (80), was prepared from pancreatic extract. It differs from the enzyme described by Waldschmidt-Leitz (81) in being activated by trypsin and enterokinase, and digesting in an alkaline medium.)

Aminopolypeptidase. A peptidase acting on tri- and poly-peptides at an optimum pH of 8. The peptide chain is attacked from the free $-NH_2$ end. Thus leucyl-glycyl-glycine → leucine + glycyl-glycine.

Dipeptidase. A peptidase acting on dipeptides at an optimum pH of 8.

Protein synthesis and hypertrophy. The problem of protein synthesis in tissues is a corollary to the conception of autolysis as a physiological mechanism operative in atrophy. Tissues normally remain without change in mass over long periods of time. Under slightly different conditions of nutrition, hormone influence, blood supply or activity, they develop atrophic changes. Under opposite conditions the same tissue returns to its normal stable mass. The stability itself is obviously one of a nicely balanced equilibrium of opposing forces. In our earlier review of the subject, we postulated a reversible mechanism controlled by a favorable blood supply and a favorable pH. Since acids were found to condition the mechanism for proteolysis by converting stable proteins into substrata, it appeared logical to assume an alkalinity about that of blood to be a medium favorable to synthesis. A protein molecule produced under these conditions would be a base-protein salt and resistant to proteolysis. The hypothesis was obviously too simple and did not suggest ways of experimental study.

The recent developments in activation and its relation to the balance between oxidation and reduction within the cell has reopened the problem and developed experimental attack. The problem derives especial interest at this time because of the attention which is being focussed upon malignant tumor growth.

The first suggestion that protein synthesis or hydrolysis is directed by the oxido-reductive system in the cell, expressed by the −SH: −SS− ratio, seems to have been made by Mothes in 1932 (82). His experimental work was done on the common onion, and indicated protein synthesis when the oxidative mechanism was maintained at high level.

Shortly after, Voegtlin and co-workers published an important series of papers on autolysis and synthesis in which rat tumor, muscle, and papain were studied. In the first of these papers Voegtlin and Maver (37) showed that in rat tumor tissue, at fixed pH levels, digestion was most extensive if the O_2 tension in the brei was low. Under these conditions the −SH reaction with nitroprusside remained high. Where the O_2 tension was elevated by bubbling air through the digest, the −SH reaction was weak or disappeared, and the autolysis was decreased. In the alkaline range from pH 6.6 to 8.0, the nitroprusside reaction was negative and there was no digestion. In the acid breis, up to pH 3, the −SH persisted, and was stronger in low oxygen tension where digestion was also more extensive. Hydrogen peroxide or Cu ions abolished the nitroprusside reaction and inhibited autolysis.

In the second paper, Voegtlin, Maver, and Johnson (83), 1932, were

able to obtain definite evidence of protein synthesis under conditions essentially opposite to those favoring proteolysis. Muscle digests were buffered to near neutrality. Cleavage was measured by protein precipitated by trichloracetic acid, and by formol titration of amino acids. A fore-period of 2 to 3 hours with N_2 bubbled through the digests demonstrated proteolysis and provided an accumulation of split products. This was followed by a period of several hours under high O_2 tension, followed by an after-period of low O_2. During the initial period of low O_2 tension, precipitable protein diminished, amino acid titre increased, and the total $-SH$ and $G-SH$ remained constant. In the O_2-high period precipitable protein increased, amino acid titre decreased or was variable, while total $-SH$ and glutathione$-SH$ rapidly diminished. During the third period of low O_2, autolysis was again resumed. Similar results were obtained with Jensen sarcoma tissue, Walker carcinoma, and with papain acting on fibrin. Changes in the precipitable protein were impressive and clearly beyond the range of experimental error. For example, in the digestion period, the protein of a Walker carcinoma fell from 63 per cent to 54 per cent of the total. When O_2 was introduced, it rose within an hour to 82 per cent. The amino acid titre is less impressive because small at pH 7, and because secondary cleavage by erepsin often partially obscures the drop associated with synthesis. A typical experiment is illustrated in figure 4, adapted from Voegtlin's data. These results were shortly confirmed by Rondoni and Pozzi (84) working with liver extracts and determining protein which was precipitated by trichloracetic acid. Cysteine in the presence of $FeSO_4$ produced active proteolysis. The reaction was reversed when the mixture was made slightly alkaline and H_2O_2 added. Blagoveshchenskii and Koram in 1934 reported similar results (85). Voegtlin has recently summarized his conception of the control of protein synthesis and hydrolysis by O_2 tension and the oxido-reduction system of the cell (86).

In attempting to summarize the conditions which now are believed to lead to autolysis and atrophy, we should emphasize the following: 1, a diminution in oxidative metabolism, which may sometimes be due simply to a diminished blood supply leading to subnormal O_2 tensions in the tissue; 2, a relative increase in the production and accumulation of acids resulting from (1), including CO_2 at times, which raise the H ion concentration and activate substrate; 3, the associated persistence of relatively high levels of protein$-SH$ and reduced glutathione, favored by low O_2 and acid reaction, which increases proteolytic activity and range; 4, removal by dialysis of cleavage products so that the reaction may continue to completion.

The conditions which favor synthesis and hypertrophy would appear to be: 1, an adequate blood supply maintaining normal O_2 tension in the tissue; 2, maintenance of a normal pH at about pH 7 + (by adequate buffer supply, prevention of CO_2 accumulation and asphyxial acid production); 3, the presence of abundant −SS− compounds and low −SH concentration; 4, diffusion into the cells of sufficient amino acids from the blood to permit the synthetic reaction to take place. It may be permissible to suggest that the newer data on activation by sulfhydryl indicates labilization of proteins not otherwise accessible to cathepsin. As is the case when the H ion concentration is increased, the presence of −SH leads to an increased digestion—to a higher level of soluble N when the reaction reaches equilibrium. Both acid and −SH there-

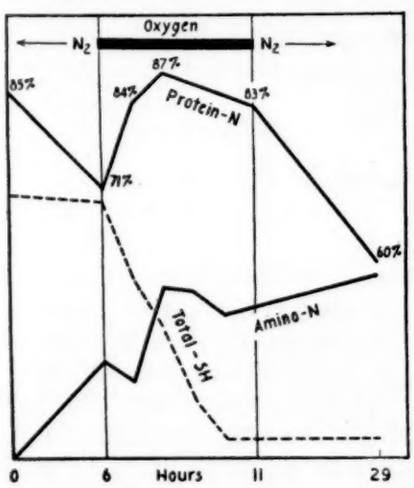


Fig. 4. Proteolysis and synthesis conditioned by the oxido-reduction balance in Jensen rat sarcoma, adapted from the data of Voegtlin, Maver and Johnson

fore are important in conditioning, or "activating" the tissue proteins for cleavage. Whether they also render the enzyme more active has not yet been clearly demonstrated. Both factors depend ultimately on the oxido-reductive balance of the cell. Thus there begins to emerge from the rather confusing and often contradictory observations of the last fifteen years the concept of a mechanism adjusted to such a nicety that small changes in pH or in the oxido-reductive system acting on the −SS− cross-links perhaps in the protein molecule, increase or decrease fragility, and so lead to atrophy, hypertrophy, or to mass stability of a tissue.

There are other factors which influence tissue growth and tissue disintegrations of which we know little. Certain hormones appear to be necessary for stable equilibria in specific tissues. When they are

withdrawn, atrophic changes set in. Thus castration of the male leads to atrophy of the seminal vesicles and prostate which can be prevented by hormone injections. Other hormones produce spectacular hypertrophies. Oestrin and progestin cause developmental hypertrophy of the resting mammary gland. The gonadotropic hormones augment the weight of the ovary several hundred fold. Its hypertrophy is also stimulated by such non-specific materials as Cu salts and yeast. Thyroid material and iodine compounds fed the tadpole produce directly or indirectly, complete atrophy of the tail, while at the same time stimulating growth hypertrophies of the limbs. The carcinogenic substances cause rapid and uncontrolled growth of certain tissue cells. As yet no mechanisms for mediating these effects have been described. It seems probable, however, that such examples of rapid tissue changes will eventually prove to be specific modifications of the fundamental processes of autolysis or its reversal.

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THE CAUSES AND MECHANISM OF THROMBOSIS

MARTIN SILBERBERG

Department of Pathology, Washington University, School of Medicine, Saint Louis, Mo.

By thrombosis we mean the intravital partial or complete obstruction of a vessel by a clot due to changes in preëxistent constituents of the blood: these may affect either cellular elements or plasma or both of these substances. Thrombosis occurs more commonly in veins, but is found also in capillaries, arteries and heart. This phenomenon is preceded by or associated with disturbances in the chemical constitution or in the circulation of the blood, with inflammatory processes or injuries to the vascular wall.

Thrombosis has been found in association with the following clinical conditions: 1, alteration in the diameter of the vessel lumen with subsequent changes in the bloodcurrent: *a*, dilatation of the vessel lumen in cases of varicose veins, aneurysms, periarteritis nodosa; *b*, narrowing of the vessel lumen following ligation, compression of the vessel from the outside or obstruction inside the vessel; 2, certain metabolic changes such as cachexia, obesity, experimental hypothyroidism, hyperparathyroidism may be associated with thrombus formation; 3, disintegration of bloodelements including polymorphonuclear leucocytes due to the action of poisons; 4, alteration in the physico-chemical balance of the bloodproteins following tissue necrosis, hyperallergy, infectious diseases; 5, modifications in the viscosity of the plasma in cholera, leukemia, hyperglobulia, chlorosis; 6, alteration of the boundary between blood and tissue due to lesions of the vascular wall, phlebitis, arteriosclerosis; supposedly in certain cases of irritation of nerves and in postoperative shock, in particular, when acting on the veins of the abdominal and genital region; 7, changes following the action of excessive heat, cold or radiation.

Thrombosis occurs commonly in adults, more rarely in children, and somewhat more frequently in women than in men. An increase in the incidence of thrombosis has been reported in recent years. Considering, however, the great differences in the character of the control material used for comparison a correct interpretation of the figures

published is doubtful and it seems that definite conclusions in regard to incidence cannot be drawn at present. Moreover, a critical review of statistics, published in earlier periods, reveals the fact that temporary increases in the occurrence of thrombosis have been noted from time to time, and it is likely, therefore, that the increase more recently observed represents one of these cyclic variations.

Also racial differences as to the susceptibility to thrombosis have been established. Reports from hospitals in China, where Europeans and natives live under the same environmental and dietary conditions, indicate that thrombosis is very much rarer among Chinese than among Europeans. Differences in the metabolic rate, which is higher in the Chinese, have been considered as etiologic factors, but here again the data are not convincing, and it will be necessary to make further observations on a larger scale, before conclusions in regard to racial differences in susceptibility can be accepted.

Appearance of thrombus. Grossly, a fresh thrombus which is not or is only slightly adherent to the vessel wall appears as a clot, round in shape and of varying length. One may distinguish morphologically the following types of thrombi: 1, red thrombus; 2, white thrombus; 3, mixed thrombus. The latter type is the most frequent and represents a combination of the first two types.

Structure of thrombus. A thrombus may show either a laminated or a uniform structure. In the former instance layers differing in color alternate. This arrangement is supposedly due to a slow clotting process, during which the bloodflow may remain partly preserved, so that a pulsation thrombus occurs. On the other hand, one may expect a uniform clot in cases in which an acute stoppage of the bloodcurrent or stagnation in the bloodflow has taken place; thus a stagnation thrombus develops. Microscopically, the red thrombus consists chiefly of red corpuscles, the white thrombus of thrombocytes and leucocytes, the mixed thrombus of all these elements. Fibrin may or may not be present.

Origin of thrombus. As to the origin of the thrombus one may distinguish a spodogenous or agglutination thrombus consisting of agglutinated cells or cell particles and a flocculation or coagulation or fibrin thrombus. An old thrombus as a rule becomes dryer as a result of gradual dehydration and resorption of its fluid constituents. Due to growth processes on the part of cells which have penetrated into the bloodclot from the surrounding connective tissue,—growth processes in which also cells which were enclosed in the thrombus or the lining vascu-

lar endothelium may participate—, an organization of the thrombus by connective tissue may take place leading to a closer connection between the thrombus and the vessel wall. At still later stages, the thrombus may become vascularized and the bloodflow may be restituted through the thrombus (canalization). Eventually, a thrombus may calcify or may be dissolved through enzymes set free in it, leading to puriform liquefaction.

Clinical significance of thrombosis. This consists mainly in local disturbances of circulation. At first there is congestion, anemia or edema of the tissue normally supplied by the thrombosed vessels. This stage may be followed by atrophic or degenerative processes in this area. In other cases, a thrombus may extend continuously through the lumen of the vessel, or multiple thrombi may develop consecutively in various places; the latter condition is called thrombosis migrans. However, the main danger of thrombosis consists in the occurrence of embolism: A whole thrombus or large parts of it may be broken off and transported into the endbranches of the pulmonary artery, occluding the lumen of larger vessels and thus leading to sudden death by asphyxia. In some instances, smaller particles of the thrombus may occlude smaller vessels and give rise to the formation of infarctions. Sometimes a transfer of the emboli into the arterial system may take place in individuals in which the foramen ovale of the heart has remained open; or, occasionally, a retrograde movement due to changes in the blood pressure within the vessels may occur. There exists in addition the possibility that a bloodclot, which provides a very good culture medium for microörganisms, may become infected and give rise to local suppuration or to general septicemia.

Thrombosis offers thus many problems which are of interest from the clinical and pathologic-anatomical aspects. But we are here concerned primarily with the physiological factors underlying the formation of thrombi.

The term thrombosis is derived from the greek term "*θρόμβος*"; it was used by Hippocrates and Galen for the description of blood-coagulation, in particular that following hemorrhages in the tissues. In his discussion of the vascular system, Diocles stated that an obstruction in the lumen of vessels may occur in the course of inflammatory processes. The original concept of thrombosis is therefore that of an intravascular coagulation process, and more than 2000 years later it was defined in a similar way by Cruveilhier, who considered a thrombus as a fibrinous exudation on the part of the vascular wall. He thus

refuted the opinion of earlier French and English investigators, in particular of Baillie and Bouchut, who believed that thrombosis and stasis of the blood were related. Rokitansky emphasized that primary inflammatory or chemical alterations of the blood itself may lead to a surplus of fibrin, a so-called "hyperinosis" or fibrin "krasis" which manifests itself in an increased coagulability of the blood. According to Virchow, bloodclots in the vessel may develop spontaneously due to local or general circulatory disturbances, and independently of bacterial contamination or chemical changes of the blood; furthermore he emphasized the significance of mechanical factors in the genesis of thrombosis.

The theories, varying through the years, concerning the etiology of thrombosis, were influenced by the more general pathological concepts predominating at the time. Hence, at some periods, chemical, at others physical hypotheses prevailed with regard to the causes and mechanism of this process.

The present review deals mainly with some of the physiological aspects of this problem which have developed in recent years.

I. Statement of the main problems in thrombosis. The majority of thrombi are associated with the precipitation of coagulated fibrin. Therefore, it is essential to decide whether or not thrombosis and blood-coagulation within the bloodvessels in the living organism are identical phenomena or differ from each other, and, if so, what their relations are and wherein they differ. As stated above, Virchow interpreted thrombosis as an intravascular coagulation and so identified both processes. Similar were the views of Weigert; and a good many investigators since have accepted this opinion, although Mantegazza and Zahn (115) had produced experimentally intravascular clots which differed from those resulting from blood coagulation; thus following injection of a concentrated solution of sodium chloride into the mesenteric veins of frogs, they found cells normally circulating in the blood attached to the vascular wall, particularly in places where vessels branched off.

The investigations of Hayem and Bizzozero (9), which revealed that the platelets represent a third element of the mammalian blood, threw new light on thrombosis. Bizzozero suggested that by disintegration of these conglomerated thrombocytes a coagulative ferment may be set free and that coagulation may follow this massing together of thrombocytes. Eberth and Schimmelbusch (25) confirmed the observations of Bizzozero that an agglutination of thrombocytes may give rise to the formation of bloodclots, especially under the influence of

changes taking place in the bloodflow. Leo Loeb (54–58, 64) proved in comparative studies that agglutination processes in the circulating body fluid may occur independently of and unaccompanied by coagulation processes in certain primitive organisms. Furthermore he (54, 55, 62–64), as well as Schwalbe (94, 95), has shown that, if in higher animals coagulation of the plasma is prevented by the use of coagulation-inhibiting substances, agglutination of the thrombocytes may remain unaffected thereby. A thrombus produced under such conditions is solely due to agglutination processes, and precipitation of fibrin is lacking. In the bloodserum the presence of coagulation-inhibiting substances was noted (54, 55, 59, 62–64).

Thus, agglutination of bloodelements as a process fundamentally different from bloodcoagulation was introduced into the mechanism of thrombosis. In the analysis of the thrombotic phenomena, one has to consider, therefore, three principles, their significance and mutual interaction:

1. The agglutination of thrombocytes, white cells and red corpuscles.
2. The coagulation of the bloodplasma.
3. The rôle of coagulation-inhibiting substances.

II. Comparative pathology of thrombosis. In a series of investigations Leo Loeb (54, 55, 60, 64) studied the comparative pathology of thrombosis. The data thereby established were of significance in evaluating the relative importance of agglutination and coagulation in thrombosis. In certain arthropods in which fibrinogen is lacking it is possible to study the pure agglutination process. The blood of Limulus polyphemus was found best suited for this purpose, as it contains but one type of cells, the amoebocyte (53–57, 60, 64). Moreover, it is possible here to analyze in vitro the process underlying agglutination. If Limulus blood is withdrawn from the animal and allowed to flow into a dish without special precautions, the normally elliptic bloodcells round off; the cytoplasm takes up fluid; the amoebocytes stick together, forming a clotlike material filling the vessels, but which gradually retracts, resembling in this respect normal bloodclots. However, it can be shown that such a clot consists merely of agglutinated cytoplasm of amoebocytes. This process represents a true agglutination due to changes which take place primarily in the hyalin ectoplasm of the amoebocytes; it is not due to the deposit of a layer of protein on the periphery of the cells. In addition, a second phenomenon can be observed: the protoplasm of many cells forms pseudopods, due to rhythmic changes in the consistency of the ectoplasmic layers which soften,

in a first phase; in other cases, the whole amoebocyte may be drawn out into long fibers; it may gain elastic properties and retract in the same manner as true fibrin does. In the living cell these changes leading to pseudopod formation are reversible. In Limulus, a pseudocoagulation based upon cellagglutination takes, therefore, the place of the true coagulation of fibrinogen in higher organisms. The coagulation-promoting explosive corpuscles which Hardy found particularly in Gammarus and Astacus, are lacking in Limulus and hence are not concerned in the pseudoclotting in this species. But also in Gammarus marinus, Tait (104, 105a, b) missed such corpuscles and emphasized the fact that cell agglutination is the only visible factor in the formation of a bloodclot.

If, instead of allowing the blood of Limulus to flow over rough surfaces into the vessel, it is collected with certain precautions, many of the changes just mentioned can be avoided. It is then possible to collect the bloodcells in vitro almost unchanged. Instead of forming a clot, which includes almost the whole fluid part of the blood and which then retracts fibrinlike, the amoebocytes round off and slowly sink down to the bottom of the vessel and here they only agglutinate to one another, forming thus a thin tissue-like layer of agglutinated cells. If small pieces of this amoebocyte-tissue are cut out and studied in tissue culture (60, 61), they behave in a similar manner to vertebrate tissues; they grow out in contact with solid surfaces and secondarily form paraplastic tissue-like structures. The amoebocyte tissue is therefore the most simple type of an agglutination thrombus and at the same time it resembles also, in certain respects, tissue.

In sponges, the conditions are in principle the same, although slightly more complex than in amoebocyte-tissue in as much as here several kinds of cells circulate in the body fluid. Among these cells Galtsoff (30a) has shown that the archaeocytes play the rôle of amoebocytes, agglutinating likewise without the aid of a protein deposit, and once the cellaggregation has been completed, more complex paraplastic structures may be produced. As far as the behavior of amoebocytes and especially the formation of pseudopods is concerned, observations similar to those of Loeb were subsequently made by Pantin and Spek and also in the case of amoebae Chambers and Mast found corresponding conditions, although certain complications may exist in various organisms.

Fredericq (29) and Heim (37) as well as Loeb (54a, 55, 57) found that true bloodcoagulation occurs first among the arthropods in certain

species of dekapodes. In particular, in Homarus americanus, Callinectes and Platyonycellus ocellatus, after periods varying from a very short time to ten hours, the cellagglutination is followed by a characteristic true clotting which in Limulus is lacking. In the crustacean blood the spontaneous clotting occurs under the influence of substances which can be extracted from the masses of agglutinated amoebocytes and which normally pass into the bloodplasma from the stimulated or injured bloodcells. These substances may be designated as bloodcoagulins. There is a second kind of substance which causes coagulation of the blood, namely, the tissue coagulins; they can be extracted from the muscle tissue of various crustaceans. The tissue- and bloodcoagulins differ from each other in degree of resistance to injurious factors, and in regard to calcium requirement in the coagulation process, which is much greater on the part of the tissue coagulins. It is only the tissue coagulins of crustaceans which induce the coagulation of crustacean blood. There exists here then a certain specific adaptation (59, 63) between tissue coagulins and the fibrinogen of the crustacean plasma. A complication arises, however, in the interpretation of these data, inasmuch as we are not able to test the effect of tissues other than those of crustaceans on their own blood. It is therefore possible to assume that tissue coagulins develop only in those species in which also coagulation of fibrinogen occurs. Furthermore, there exists in addition a certain specific adaptation between the bloodcoagulins and the crustacean plasma. While the existence of a specific adaptation (63) between bloodplasma and tissue coagulins and the lack of it between bloodcoagulins and bloodplasma is pronounced in the case of vertebrate blood, such a relation cannot be demonstrated in the case of invertebrate blood. However within a wide range a specific adaptation of tissue coagulins can be demonstrated also in the case of invertebrate blood and it may exist perhaps even within more narrow limits. But in both invertebrate and vertebrate bloodcoagulation, there is evidence that tissue coagulins and blood coagulins are not identical and in the case of invertebrate blood it can moreover be shown that tissue coagulins cause the transformation of fibrinogen into fibrin directly and not indirectly by their action on a precursor substance of the thrombin (53–55). Such a direct action of tissue coagulin on fibrinogen is probable also in the case of vertebrate blood.

If we now turn to the blood of vertebrates, we may distinguish the lower classes, in which the place of amoebocytes is taken by nucleated spindle cells, and the mammals, in which the cytoplasmic parts of

megakaryocytes, the bloodplates, function in the same way. Avian blood—geese and pigeons as the representative of the former (54a, b) —may remain unclotted for some time outside the body, if it is protected from contact with tissue elements; microscopically, clumps consisting of non-hemoglobin containing mononucleated cellular elements, which have agglutinated with one another, are seen adhering to the wall of the vessel or to the glass slide on which the blood was collected; this experiment indicates that also in vertebrate blood agglutination of cellular elements may precede coagulation and occur independently of the latter, although similar factors may be effective in inducing or accelerating these two processes.

Studies on blood of rats, cats, guinea pigs and rabbits disclosed similar conditions. If bloodplasma of guinea pigs (53–55) is diluted and centrifugalized, and coagulation is prevented or delayed by heating to a certain temperature, the blood platelets are seen to agglutinate, and sink to the bottom of the slide to which they stick. As in arthropods and the blood of lower vertebrates, an agglutination of cellular elements of the blood precedes the coagulation process and occurs independently of the latter. A subsequent addition of tissue extracts or of particles of a bloodclot causes coagulation of the plasma. Special attention was given in these experiments to the effect of pieces of the vascular wall, and extracts from such tissues, but their action was not different from that of other tissues and in particular no inhibiting effect on coagulation was detected.

Throughout the various classes of vertebrates a specific adaptation can be demonstrated between the tissue coagulins and the coagulable bloodplasma. The coagulative effect is greatest and most rapid, if tissue coagulins and plasma belong to the same class of animals. Tissue coagulins can be extracted also from mammalian and presumably also from other kinds of erythrocytes; here a specific adaptation can be demonstrated even between certain mammalian species. In addition to the specific adaptation between the tissue coagulins and the bloodplasma, there exists also a specific adaptation between the inhibiting substances present in the bloodserum and the tissue coagulins in their action on bloodplasma (59, 63); the inhibition is greatest, if bloodserum, tissue coagulin and plasma belong to the same class or order of animals.

These comparative investigations have shown then, that during the phylogenetic evolution of animals two processes developed side by side by means of which the organism reacted against interferences with the various constituents of the blood or body fluids, namely: 1, an agglu-

tination of cellular elements, and 2, a conversion of a colloid constituent of plasma, the fibrinogen, into the coagulated fibrin. The first one of these two processes developed earliest; it is the only one existing in the more primitive organisms; in the higher organisms there was adde to this the second one, the coagulation process; but even in the highest organisms the first, the agglutination process, persists and precedes coagulation. In invertebrates it is the amoebocytes, in the lower vertebrates the spindle cells and in mammals the bloodplates which primarily react by means of agglutination and all these cellular elements show a similar mode of reaction to certain environmental changes. These two processes, agglutination and coagulation, represent therefore coördinated processes and while in lower organisms the former is present alone or is much predominating, in the higher classes of animals the coagulation process becomes more and more accentuated.

The agglutination of the amoebocytes, which can be studied in lower invertebrates in vitro, can also be induced experimentally within the animal, for instance by introducing threads into the bloodchannels of Limulus; the amoebocytes agglutinate around the rough surface of a thread and stick to it in the same way as they agglutinate outside the body. This agglutination process which takes place outside the body and which may be induced also in the animal body is the first stage in thrombosis, and in higher organisms it is followed by a coagulation process.

III. The mechanism of cell-agglutination. Three theories have been advanced to explain the mechanism underlying agglutination of cells and in particular of the cellular elements of the blood. 1. The first theory assumes that agglutination is due to changes in the ectoplasmic layer of the cells, which makes them sticky. The processes leading to agglutination are identical with or closely related to the processes that lead to amoeboid movement of these cells or parts of cells. In amoeboid movement, a rhythmic wave-like softening and hardening of the ectoplasmic cell layer occurs, in which probably changes in the fluid content and viscosity of the protoplasm play a rôle. In agglutination it is the first phase of amoeboid movement consisting in a softening of the ectoplasmic layer, caused by an increase in the fluidity of this substance, which is the essential factor. This theory then assumes that agglutination and amoeboid movement are closely related processes.

2. According to the second theory agglutination is due to changes in the electric charge of the surface of the cells which prevent the mutual

repulsion of particles carrying the same electric charges. If this change has taken place, other factors, e.g., action of certain electrolytes, may lead to agglutination.

3. The third theory assumes that a protein substance, which is deposited on the surface of the cells, makes the cells sticky and leads to their agglutination.

We shall now weigh the evidence in favor of these three modes of interpretation.

1. The conditions under which agglutination of the thrombocytes and of the cells reacting in an analogous manner occurs, can most conveniently be studied in vitro, if we observe the reactions of amoebocytes to various physical and chemical changes (60). We then find that in the living normal animal the amoebocytes, as far as structure and modes of reaction are concerned, resemble the spindle cells in the blood of the lower vertebrates; the elliptic discs neither stick to the wall of the vessels nor to one another. But physical changes such as those induced by contact with rough surfaces suffice to make them send out pseudopods and to become sticky, so that they adhere to the surface of the glass and to one another. Changes in the surrounding medium, such as variations in the hydrogen and hydroxyl ion concentration, preponderance of certain kations and anions of inorganic salts, changes in osmotic pressure of the surrounding fluid, the action of organic substances functioning as non-electrolytes all indicate that various factors affect in a similar way amoeboid movement and agglutination. Those conditions that tend to harden the ectoplasmic layer and withdraw fluid from the cells counteract agglutination; they either diminish or prevent it and they likewise diminish or prevent amoeboid movement; if amoeboid movement still occurs, the pseudopods which are formed under these circumstances are sharp and thin. If the hydrogen ion concentration reaches a certain degree, it causes a hardening of the cell and especially of the ectoplasmic layer, and the cells that were agglutinated may separate again. Agglutination under these conditions is a reversible process. Those kations and anions that tend to cause a swelling of gelatin, soften the amoebocytes and favor, therefore, within certain limits, agglutination and amoeboid activity; however, if applied in too strong a concentration, they may cause too great a fluidity of the cell and thus interfere with amoeboid movement. Hypotonicity of the surrounding medium acts in a similar manner. The structural changes which can be observed under these conditions indicate a taking up of fluid by the amoebocytes. Amoeboid movement itself is associated

with and indeed depends upon a stickiness of the ectoplasmic layer of the pseudopods and also of that of the remaining cell; without these changes locomotion would be impossible. There is then a close association between the mechanism underlying amoeboid movement and agglutination of cells, as far as the inciting, as well as the underlying, cause is concerned. They are related phenomena which depend upon changes in the consistency, state of contraction and presumably the fluid content of the ectoplasmic layer. On the basis of these observations Loeb (60, 64) concluded that agglutination of the amoebocytes depends upon alterations which occur in the ectoplasmic layer of the cells, as reactions to certain environmental stimuli or under the influence of changes in the medium, which influence the colloidal state of the ectoplasm. It may be assumed that conditions are similar in the case of the spindle cells of lower vertebrates and of the mammalian thrombocytes.

2. The second theory of the mechanism of agglutination is based on experiments by Bechhold (6), Friedemann (30) and Michaelis (70, 71) who studied the agglutination process by using bacterial suspensions and agglutinating sera.

Under normal conditions, the red corpuscles and the thrombocytes have a negative charge and therefore repel each other, remaining in suspension. It is assumed that agglutination is induced by a loss of the electric surface charge. Such a discharge is supposed to occur as a result of adsorption of globulin and fibrinogen by the surface of the cells, which takes place the more readily, the closer the isoelectric point of the particular protein approaches the neutral point. An increase of the globulin-fibrinogen-fraction, as is found in certain diseases, is supposed, therefore, to lead more readily to agglutination. Red corpuscles are less agglutinable than the thrombocytes, because they have normally a higher electric charge.

As far as thrombocytes are concerned, the findings of investigators who attribute their agglutination to changes in their electric charge (Stuber, 102, 103; Schulte, 93; Zschau, 118) are open to various interpretations. This applies also to the conclusions of Bechhold (6), Hoeber (40), Schade (92), Abramson (1), Mainzer (65), Woehlisch (111). In the experiments of different investigators the technique of withdrawal of blood, the time of observation or the temperature at which the observations were made differed. Thus the experimental conditions in the case of different species varied in these instances and conclusions based on in vitro findings cannot be properly applied to conditions holding

within the living body. If, as has been stated by Schulte (93), agglutination of thrombocytes occurs in an isotonic salt solution free of colloids at pH 3.5 to 4.3, this would correspond to a degree of acidity which is never found within the living organism. The same objection holds good as to the figures concerning the isoelectric point of thrombocytes as determined by means of cataphoresis; here different determinations vary as much as pH 3.49 to 4.7 (42, 65, 102).

Furthermore these conclusions are based on the assumption that changes in surface tension of the cellelements cause their adherence to the vascular wall; this assumption implies that these cells are liquid in consistency and that their changes in form are due merely to changes in the surface tension of a fluid system. However as we shall discuss later, the structure of these cells and the changes they undergo under various environmental conditions are more complex than is compatible with the assumption of a fluid state of the cell. The surface of the amoebocytes is not a fluid surface film, which merely undergoes changes of the surface tension, but it approaches a solid state and under various conditions changes in consistency. In the case of normal amoeboid movement, these changes are of a rhythmic, wave-like, reversible character. This holds good in general, although under certain circumstances the surface changes may be of such a nature that a liquid state is approached, and only in this case might changes in surface tension, corresponding to those that occur in drops of liquid, explain the behavior of these cells.

Lastly we may point out that conditions are not the same in the case of agglutination of bacterial suspensions on the one hand and of amoebocytes, spindle cells and thrombocytes on the other. In the latter case, agglutination occurs between the cells, e.g., the amoebocytes and all kind of surfaces, even paraffin or vaselin, irrespective of the natural electric charge of these surfaces. Agglutination may take place even in the autogenous plasma and serum provided certain mechanical factors are made to act on these cellular constituents of the blood. Moreover, these cells do not agglutinate in the form in which they are present in the blood as spindle cells or elliptic plates, but previous to agglutination they undergo changes in form or consistency which correspond to those which are characteristic of the first stage of amoeboid movement.

3. According to the third theory agglutination is due entirely to a precipitation of protein, in particular of serumalbumins or to a swelling of certain intercellular proteinsubstances which are deposited on the surface of the cells; lipoids may co-operate. It seems however that this

phenomenon has been observed only in cases in which protein was set free from dead cellmaterial. In some instances, however, a conglomeration of cells is found as a result of fibrin deposition following coagulation processes. This passive type of agglutination is, therefore, a phenomenon secondary to coagulation processes and depends upon the latter, while it can be shown that the agglutination processes which typically initiate thrombosis precede and are independent of coagulation processes. Furthermore, the experiments with amoebocytes reveal that protein deposits on the cellperiphery are not necessary in agglutination and that in normal amoeboid movement, a process, which as noted above, is closely allied to agglutination, a deposit of protein does not play any rôle.

4. Agglutination-thrombi. Various types of cells or part of cells can enter into agglutination thrombi. Accordingly we distinguish several types of agglutination thrombi.

a. *Platelet-thrombus.* As stated, the phylogenetically oldest type of thrombosis, which has been reproduced in the living organism, is that in Limulus observed by Loeb (55d, 60a) after introduction of foreign bodies into the blood-lymphchannels of this animal; this process represents the prototype of a pure agglutinative thrombus. It could be shown that the amoebocytes behave in exactly the same way within the body as they do in vitro: they become sticky, agglutinate and form a tissue like structure. However, the more highly developed a species is, the more rarely one encounters a pure agglutination thrombus. In Amphibia, Samuel (91), Wharton (110), Zahn (115) noted that physical irritations, particularly heat, if applied by means of a warm water bath, may cause an agglutination of the spindle cells in the vessels of the tongue and the mesentery; the axially located spindle cells are tossed toward the periphery of the vessel and agglutinate here on the vascular wall, especially where branches are given off by the larger vessels. In mammals, agglutination of thrombocytes has been seen in the web of bats and in the mesentery of rabbits and dogs, in certain instances as the first sign of thrombosis (Samuel, 91; Zahn, 115; Bizzozero, 9), independently of any precipitation of fibrin. In the vessels of the mesentery or in the jugular vein, after application of mechanical irritations or the introduction of wettable foreign bodies, one may see an agglutination of thrombocytes which covers the surface of the foreign body within a few minutes, especially if simultaneously a certain retardation of the bloodcurrent has been induced. When irritating chemical compounds, such as silver nitrate, Lugol's solution, carbolic acid, croton

oil or turpentine, are applied on the outside of the vessel wall, and the endothelia of the capillaries are thus damaged, conditions are created which favor an adherence of thrombocytes to the injured wall as well as subsequent agglutination of the bloodplates to each other. But such an agglutination may occur even in certain instances in which the above named substances are applied intravascularly, without any lesion of the vessel wall being visible.

Such thrombi are found particularly in the pulmonary capillaries or capillary veins, or in the veins of the spleen, in areas, therefore, in which the bloodcurrent is relatively slow. If, however, a complete stoppage of the bloodflow by means of a double ligature is induced, thrombosis does not occur, whereas it can be demonstrated in cases in which only one ligature had been applied. Therefore, the bloodcurrent has to be maintained to a certain extent in order to provide a permanent supply of the necessary cellular elements.

The process underlying this kind of thrombosis has been analyzed experimentally by means of anticoagulants. Schwalbe (94) and Loeb (54, 55) first used this method independently of each other. Loeb ligated and tore the vessel wall of warmblooded animals in which hirudin had been previously injected. He demonstrated the formation of platelet-thrombi in dogs and geese. Under these conditions fibrin formation had been made impossible; yet the agglutination of bloodplates had not thereby been prevented. In dogs, injection of hirudin, in sufficiently large amounts, could retard or diminish also the agglutination process, but in geese agglutination was not affected under conditions in which bloodcoagulation had been entirely inhibited. By injections of homologous serum, which also contains coagulation-inhibiting substances, Loeb (54e) obtained similar results. Schwalbe again showed that after cauterization of the vessel following injection of an anticoagulant the bloodflow has to be maintained for the production of thrombi. The work of Loeb and Schwalbe has since been repeatedly confirmed, in particular by Yatsushiro, Derewenko, Kusama, and more recently by Johnson and Shionoya (44, 96) who, in accordance with a method described by Rowntree (88), severed the vessel and interposed a dialyzing cellophane loop between the two ends of the bloodvessel, thus permitting observations of the bloodcurrent in the living animal. They noticed that, under the influence of hirudin and heparin, thrombi were formed in the cellophane loop with some delay, as compared with experiments in which hirudin or heparin were not used. These thrombi consisted of agglutinated thrombocytes, while a deposition of fibrin was lacking

in early stages. Best (8) and co-workers (76) who quite recently repeated the latter experiments with the use of heparin and the cannula of Rowntree (88) also found that, while a deposition of fibrin may be prevented under these circumstances, agglutination may remain unaffected. In other cases they found similarly to Loeb (55a) that heparin may retard not only coagulation but also the agglutination process. These experiments confirm the conclusion that in more highly developed organisms agglutinative thrombi may originate independently of coagulation processes in the plasma, and that from the lower invertebrates on to the highest vertebrates the mechanisms underlying thrombosis are essentially the same.

b. *Red corpuscle-thrombi.* In addition to the thrombi consisting of agglutinated spindle cells or thrombocytes there occur also agglutination thrombi which are composed primarily of agglutinated erythrocytes. The mechanism underlying this type of thrombosis is similar to, but not identical with that applying in the case of thrombocytes. The agglutination of red corpuscles may occur independently of an agglutination of the bloodplatelets. It is especially certain chemical substances which affect the surface of the erythrocytes and thus produce their agglutination. The mechanism underlying an agglutination of erythrocytes in the blood may, therefore, be different from that causing the agglutination of thrombocytes, and the experimental conditions under which the former has been produced, indicate that such differences exist (26).

Landois (52) observed that after the injection of foreign serum the small pulmonary vessels were occluded by agglutinated red corpuscles which were intermingled with fibrin fibers. Flexner (28) who injected intravenously into rabbits dogserum, ether and ricin found in the right heart and pulmonary vessels thrombi, consisting of agglutinated red bloodcorpuscles which had subsequently undergone secondary hyaline changes. Loeb (54d) and co-workers were the first to produce thrombi consisting definitely of agglutinated red corpuscles, by injecting ox-serum intravenously into rabbits under conditions in which participation of fibrin formation could be excluded. Within a few minutes, a fatal agglutination of the erythrocytes took place in the pulmonary vessels causing asphyxia which led to the death of the animals. The same effect was obtained, when simultaneously with the ox-serum hirudin was injected in doses sufficient to prevent coagulation of the blood. It may therefore be concluded that it is solely the agglutination of red corpuscles without an accompanying production of fibrin which causes

the occlusion of the vessels and obstruction of the bloodflow. Coca (16) obtained similar results, when rabbits previously immunized with gooseblood were reinjected with the latter. Under these conditions, the small pulmonary arteries and capillaries were found occluded by agglutinated goose corpuscles. This agglutination was chiefly due to the agglutinins acting on the strange erythrocytes which were present in the blood of the rabbit as the result of the preceding injection of these cells. In more recent investigations, Coca (17) attributed the death of animals following the re-injection of foreign serum to a constriction of the pulmonary vessels independent of thrombosis. However, it must be noted that in the latter experiments as well as in the earlier ones with goosecorpuscles, the rabbits had been previously injected with serum and were therefore sensitized against this substance, and he did not carry out experiments in animals not previously sensitized to foreign sera. Coca's experiments do not, therefore, throw any light on the problem under consideration. The same objection applies to the experiments of Kusama (48) who attributed the death of rabbits sensitized with homologous serum to an increased viscosity of the blood which may lead to a stasis in the smaller vessels, without however giving any proof that such a change in the viscosity of the blood actually existed. On the other hand Zinsser (117) showed in in vitro experiments that goatserum hemolyzes and agglutinates red corpuscles of rabbits. The autopsy findings in experiments in which he injected goatserum into living rabbits disclosed the presence of coagula in two cases in the heart of the rabbit. Zinsser believed that hemagglutination and coagulation could be excluded as the cause of death because in the majority of these rabbits signs of coagulation were lacking. Rabinovitch (85), who repeated the experiments of Loeb (54d) with injection of ox-serum but used heparin instead of hirudin as anticoagulant, confirmed the findings of Loeb. After intravenous injection of ox serum, the pulmonary vessels became occluded by agglutinated red corpuscles; in this case it could be shown that agglutination had taken place during life and thus had led to thrombus formation. This was accomplished by removing thin slices of lung rapidly after the injection of the blood-serum, before secondary changes could have taken place. By exerting gentle pressure on pieces of lung of the injected rabbits he could squeeze out numerous thrombi which consisted of agglutinated red corpuscles. Rabinovitch concluded, therefore, that constriction of the vessels, to which Coca (17) had attributed the lethal effect, could be at best only of subsidiary significance as the cause of death. These investigations

confirm, therefore, the conclusion that hemagglutination may occur as a primary process in thrombosis independently of a coagulation process. This type of thrombus consisting of agglutinated erythrocytes and produced in the living animal is in accord with what is known concerning the agglutination of red corpuscles in vitro.

It is necessary to distinguish the problem as to the nature of the thrombi which are found in the pulmonary vessels following intravenous injection of foreign serum from the question as to the factors which bring about the death of animals under these circumstances: While it is very probable that the agglutination thrombi, produced under these circumstances, were the essential factor causing the death of the animals injected with ox serum, it is possible that in addition serum may exert certain toxic effects of a different nature, and that the sera of different species of animals may behave differently in this respect.

c. *White cell-thrombi.* The occurrence of a true agglutination of white cells in the living organism has not as yet been proven. In the course of other types of thrombosis, however, a secondary inclusion of white cells in the thrombus usually takes place. Under these conditions some amoeboid white cells, in particular the leucocytes, stick to the agglutinated clumps of thrombocytes and red corpuscles. But it is the agglutination of the latter which has to be considered as the essential factor. It is possible that, in addition to this passive inclusion, also a movement of white bloodcells may be elicited by chemotactic influences exerted by the agglutinated cellmasses. The chief function as far as the mononuclear white cells are concerned consists in their participation in the organization of the bloodclot, while the polymorphonuclear leucocytes may help in the liquefaction of the clot; the rôle of these cells in thrombosis is, therefore, a secondary one.

IV. Mechanism of coagulation. As stated above, the primary agglutination processes in the formation of thrombi are as a rule followed by coagulation processes, and particles of clotted blood form, therefore, important constituents of thrombi. After stating briefly the principal views concerning the conditions which are concerned in coagulation of blood in general, we shall consider mainly these factors which are responsible for intravascular coagulation processes, subsequent to the primary processes of agglutination in thrombosis. As stated above, these coagulative changes as a rule exceed greatly in quantity and pathological significance the primary agglutination. Following this analysis we must study the factors underlying the formation of a primary coagulation thrombus which occurs under certain conditions.

1. *Coagulation-promoting factors*: a. According to the views of Morawitz (73), kinase, set free by disintegrated cellular elements, in particular by thrombocytes, acts in the presence of calcium as an activator of thrombogen and prothrombin. Thrombogen is preformed in the plasma. As a result of these processes thrombin is produced which then transforms fibrinogen into fibrin.

b. A physical-chemical theory assumes that fibrin is chemically identical with fibrinogen and represents merely the irreversibly dehydrated gel state of the latter substance; it has also been assumed (102) that an increase of glycolysis runs parallel with acceleration of the coagulability. Others, such as Waldschmidt-Leitz (109) and Woehlisch (111, 112) assume that the fibrinferment is closely connected with the protein splitting ferments. The significance of these theories has recently been reviewed by Howell (42).

c. Wooldridge (113) considered coagulation as an interaction between a fibrinogen of type A present in the blood, the specific bloodfibrinogen, and another one of type B, a general fibrinogen, found in tissues. In investigations on invertebrates and vertebrates, Loeb (53–57) has shown that the tissues contain substances which may act as coagulins directly on fibrinogen, inducing its coagulation. The effectiveness of these substances was diminished by heating to 56° or 60°C. They are, therefore, not entirely heat stable (54b). There exists a definite relationship between the amount of tissuecoagulins and the acceleration of coagulation which they produce (55b, d). The tissue coagulins are specifically adapted to the constituents of the plasma with which they interact (59b). These findings were confirmed by Hewlett (39), Muraschew (75) and Nolf (78). The question as to whether these tissuecoagulins were identical with the bloodcoagulins aroused some controversies. While Nolf emphasized the identity of both substances, Loeb (54a, 55c, 62, 63) found distinct differences. There is no specific adaptation demonstrable within the bloodcoagulins, while this is present in tissuecoagulins, a fact confirmed by Nolf himself. Furthermore, the rôle of calcium is different in the two instances: After calcium has once combined with prothrombin to form thrombin, it is no longer needed for the action of the latter, whereas in the case of tissuecoagulins, the presence of calcium is required during the time, when tissuecoagulins act on plasma or fibrinogen.

As to the nature of the tissuecoagulins, Loeb supposed that proteins are involved, because of the specific adaptation which they exhibit to their substratum, which suggested a similarity to the substances con-

cerned in the presence of immunization. When it was found by Bordet and Delange (11, 12), Howell (41, 42), and Zak (116) that certain lipoid substances present in various tissues may act as coagulative agents, Loeb (63) believed it possible that these tissuecoagulins represent combinations of protein and lipoids. Mills (72) Kraus and Fuchs (47), and Fischer (27) concluded that tissue extract contains two agents, namely, *a*, a lipoid substance, cytozym, and *b*, a substance identical with the active substance present in the blood, prothrombin. The latter heat labile substance, when changed into thrombin, does not act as kinase, but converts the fibrinogen directly into fibrin.

In vivo, any process which leads to a liberation of coagulation-promoting substances may, under certain conditions, give rise to the formation of thrombi or increase the size of primary agglutination thrombi. As far as thrombosis is concerned, the following factors leading to coagulation have to be considered:

a. An increase of the globulin-fibrinogen fraction within the blood. This process which goes hand in hand with a decrease of bloodalbumin, is observed under conditions in which tissue necrosis occurs or in association with protein disintegration in fever (87); also, in general marasmus, and in various intoxications (7, 23, 24); it has also been associated with the rather controversial state of vagotony (101). On the other hand, in benzol or lead intoxication, in scurvey, thrombopenia, and in hemophilia (7, 86, 100) when the bloodalbumin is found to be normal, or in jaundice (44), when it is augmented, a disposition to thrombosis is not noticeable.

b. Extraction of bloodcoagulins from disintegrated agglutinated thrombocytes or from fibrin.

c. Extraction of tissue coagulins from disintegrated red corpuscles, from injured vessel walls or from the surrounding muscle tissue (54, 55c, 82). Substances causing hemolysis are important in this connection. They may set free large amounts of tissue coagulins from disintegrated red corpuscles. Foreign bloodserum, acid bile salts, certain snake venoms, ether, ricin, glycerine, toluylendiamin, carbonmonoxide, carbolic acid, ammonia, potassium chlorate and other substances may act in this manner. In all these instances, however, one has to deal with a very complex mechanism. If the substances are injurious solely to the red corpuscles, thrombosis may not occur, although the coagulability of the blood may be increased. But thrombosis is effected if substances are set free from the damaged vessel wall and from the surrounding tissues; in this way more active tissue coagulins are liberated.

d. Parenteral administration of proteins as such, but especially after a preceding sensitization, furthermore, intravenous injections of other colloid substances, and also microörganisms or substances set free by their disintegration, may cause intravascular coagulation. As far as bacteria are concerned, different types of bacteria differ in their effectiveness: Staphylococcus pyogenes aureus exerts a very pronounced coagulative action, B. coli, pyocaneus, prodigiosus, of hog cholera, and pneumococcus are less active in this respect, while B. diphtheriae, xerosis, of typhoid, tuberculosis, and the streptococcus are apparently ineffective (Loeb, 54b, 59a; Flexner, 28; confirmed by Much, 74; Dietrich, 22). The marked differences which exist between the effectiveness of different types of microörganisms exclude the possibility that the microörganisms act merely as foreign bodies in inducing coagulation and thrombosis, but they indicate that products of their metabolism represent the active agent.

e. Foreign bodies, in particular those with a rough and wettable surface, such as threads of silk, horsehair, cotton, metals and agar, also act as agents which may promote intravascular clotting and increase the size of thrombi. However, it is doubtful whether unaided by other factors they can induce coagulation in a sufficient degree to give rise to a noticeable thrombosis, but they may cause a slight primary agglutination process and determine the place where coagulation first takes place and from which it spreads.

2. *Coagulation-inhibiting principles.* From the fact that under normal circumstances the circulating blood does not clot, Bruecke (14), Klebs (45) and Dastre (18) concluded that the intact bloodelements, endothelia of the vessels or cells of the surrounding tissues may furnish anticoagulative or fibrinolytic substances, and that conversely a lack of such substances may help to induce coagulation of the blood. However, the presence of such an element within the endothelial system has not been proven. The endothelial layer of the bloodvessels probably acts merely in a negative manner; similarly to a layer of paraffin or oil, it prevents changes in the surface of the cellular constituents of the blood which might lead to agglutination, as well as changes in the colloids of the bloodplasma which might initiate coagulation of the blood. Furthermore, the endothelial lining of the bloodvessels prevents the liberation of tissue coagulins from the surrounding tissues. Three kinds of anticoagulative or coagulation-inhibiting principles exist: *a*, a constituent present in the bloodserum; *b*, substances foreign to the body; *c*, fibrinolytic substances within various organs.

a. Under certain conditions, the addition of bloodserum to a mixture of tissue extract and bloodplasma may lead to a marked delay of the coagulation; this effect increases with increasing incubation of the mixture (54, 62, 63). In many cases, the tissue coagulin may thus be made entirely inactive. In the serum of dogs, poisoned by phosphorus (54c), these inhibiting substances are lacking in the serum. There exists a specific adaptation between the inhibiting substance and the plasma and tissue extract. Loeb (63) suggested that the inhibiting substance may be a protein and lipoid combination, analogous to the constitution of the coagulation-promoting substances. It seems that insufficient attention has been given to the existence of an anticoagulative constituent present in the serum, a conclusion which has been supported by the more recent work of Howell (42) and Eagle (23, 24).

b. Among extraneous substances which have anticoagulative properties, those known longest are Witte-Pepton in certain dilutions and hirudin; also some snake venoms (7, 23) and particularly heparin have been found to have the same action. The effects differ, however, to some extent, within various species. Bordet (10, 13) and Gengou (13) assumed a counteracting or a neutralizing action of hirudin on fibrin ferment or on the precipitation of fibrin; on the other hand, it has been suggested that a diminution of the inner friction between the cellular bloodelements, together with an increase in the resistance of the thrombocytes and red corpuscles to injurious factors, might prevent hemolysis. Although these substances, because they inhibit coagulation, are very suitable for the demonstration of a pure agglutination-thrombosis, they may, as already mentioned, at the same time, under certain conditions, exert also an inhibiting effect on the process of agglutination.

c. In addition there exist fibrinolytic substances in various organs (63).

3. Experimental fibrin-thrombosis. After the injection of foreign blood or of hemolyzed blood from the same species, of ether or bile acid salts, Naunyn (77) observed in rabbits and cats the occurrence of fatal bloodclots which filled the heart and pulmonary vessels. The mechanism underlying the formation of these thrombi is evidently different from that of agglutination thrombosis. In rabbits, Loeb and collaborators (54d, e) produced pure fibrin thrombi by injections of dog serum or other hemolytic agents. The animals succumbed subsequently to the occlusion of the pulmonary vessels by fibrin thrombi. If hirudin was given with the dog serum, bloodcoagulation was inhibited, and the death

of the animals was delayed or in a number of cases even prevented. Conversely, the death of the animals and the formation of the fibrin thrombi occurred the faster, the greater the amount of serum which had been injected. The development of these thrombi could be observed directly previous to the death of the animals by examination of the lung. On the other hand, the lethal effect was absent, when, as a result of repeated injections, the animals had been previously immunized against hemolysins of dog serum. Rabinovitch (85) showed that also by the use of heparin the formation of fibrin thrombi can be prevented, while it has no effect on agglutination thrombosis produced through the injection of ox serum. It is also possible to produce fibrin thrombi in other organs than in heart and lungs: Injections of ether into the mesenteric veins of rabbits caused the formation of thrombi, which were the more extensive the larger the amount of ether injected (54e). These thrombi, which extended into the ramifications of the portal vein in the liver, consisted of fibrin which enclosed in its meshes red corpuscles; they were not hyaline agglutination thrombi. If previous to the injection of ether, hirudin had been injected, these fibrin thrombi were not obtained. The coagulation processes, which are thus induced by various means, are enhanced, if simultaneously with the action of these substances the bloodflow is retarded or if the blood comes in contact with rough and wettable surfaces.

V. THE RÔLE OF MECHANICAL FACTORS AND OF THE ENDOTHELIUM IN THROMBOSIS. Some investigators suggested that the formation of thrombi is determined solely by certain simple mechanical factors. Thus Aschoff (3) and Beneke (7), who studied the influence of mechanical obstacles on the flow of water, demonstrated the occurrence of whirls, eddies and cylinders together with a retrograde movement of the fluid. They assumed that in an analogous manner obstruction within the bloodstream might initiate irregularities in the bloodcurrent with subsequent agglutination of cellular elements or coagulation of the plasma. These experiments do not, however, explain various facts known regarding the etiology of thrombosis, although they may serve as an explanation of a certain structural peculiarity which is found in thrombi, namely, the occurrence of layers in the clot.

In 1819 Thackrah (107) reported the following interesting experiment: Two ligatures were placed around the jugular vein of a living dog and left there for three hours, during which time the blood had not clotted. Bruecke (14) extended these investigations and showed that, if in turtles, carps and other coldblooded animals vessels are ligated in

two places, the blood contained in the ligated segment may remain liquid for a certain length of time. In warmblooded animals, the delay in coagulation in ligated bloodsegments was much less than in cold-blooded ones. These observations have been confirmed by Baumgarten (4, 5) who emphasized that coagulation can only be prevented, if an injury to the vascular endothelia is carefully avoided.

It follows from these experiments that stoppage of the bloodflow, as such, cannot be considered a sufficient cause of thrombosis. It is, therefore, probable that for the formation of thrombi a steady movement of cellular elements of the blood along a certain place in the vessel wall is essential; however, if the bloodflow is too strong, conditions are not suitable for the formation of thrombi, because, owing to the force of the current, the first deposits may be pushed away from the wall of the vessel. The most favorable condition is, therefore, represented by a certain retardation or oscillation in the normal bloodcurrent.

But in addition to this change in the rapidity of bloodflow, damage to the vascular endothelia is of etiological significance. Obviously, injury to the vascular wall is effective 1, because it induces liberation of tissue coagulins, and 2, because lesions to the endothelial lining expose the cellular blood constituents and the bloodcolloids to contact with a rough surface layer of the cells leading to agglutination and conditions in the bloodplasma which initiate coagulation.

In a series of investigations, Ritter (87) attempted to prove that intravenous injection of vital dyes, of protein substances or microörganisms may sensitize or irritate the layers of the vessel wall. The cells of this latter tissue become swollen and mobilized; they may disintegrate and their hydrogen ion concentration may change; he takes as an indication of the tissue changes in general the appearance of a positive oxydase-reaction. Thus colloid chemical alterations in the boundary layer separating blood and tissue, similar to those seen in aseptic inflammation, are induced. This disturbance of the physicochemical equilibrium, associated with changes of the blood viscosity, is supposed to represent a necessary prerequisite for the occurrence of thrombosis, and it is supposed to act by inducing adsorption of cellular elements or a precipitation of coagulated material, such as fibrin, on the vessel wall. If, now, to either vital dye storage or sensitization of the tissues or to both these conditions, an irritation or stenosis of a vessel or a paravascular infection or a combination of these factors was added, thrombosis could be successfully initiated. The views of Ritter have been confirmed by Dietrich (21, 22b, c) in experiments in which

rabbits previously sensitized with protein substances or bacterium coli were re-injected with these substances. If, after narrowing the lumen of the jugular vein by means of a ligature, the same kind of living or dead bacteria as had been used for sensitization was re-injected, the formation of thrombi was observed. The experiments of Dietrich are therefore similar to those reported by Ritter, as far as procedure and interpretations are concerned.

The effects noted under these conditions are probably due to an experimentally induced increase of the globulin-fibrinogen-fraction of the bloodplasma and the general assumption that an increase in the reactivity of the vascular endothelia is the only etiologic factor in thrombosis has not been proven by these experiments. Moreover, any considerable ability of the vascular endothelia (98) to act as phagocytes has not so far been demonstrated in a convincing manner. In a corresponding manner we know that the cytopoietic potencies of the vascular endothelia (98a) are likewise limited; and lastly, there is the objection that in some of these investigations the blood vessel endothelia were not sharply distinguished from the histiocytes. There is no reason, therefore, at the present time, to attribute a significant rôle to the activity of the endothelia in thrombosis, and instead the possibility of a direct effect of the increase in the fibrinogen of the blood in these experiments has to be considered.

Laker (49) and Klemensiewicz (46) maintained that injury to the vessel wall is regularly followed by the precipitation of a fibrinous gelatinous membrane, which represents the first change and the central factor in every case of thrombosis, and that this membrane formation may take place independently of an agglutination of thrombocytes. Following cauterization of the bloodvessels in salamanders, Klemensiewicz described a deposition of such a delicate fibrinous mass in the form of threads, filaments and bands adherent to the intima and he noted that, if spindle cells move by such a place, they become entangled in the fibrin fibers, aggregate there and stick together; agglutination in this case would, therefore, be a passive and secondary process. In warmblooded animals, a similar precipitation of a hyalin veil was seen in cases in which changes in the intima had taken place; here, the subsequent aggregation of thrombocytes was likewise considered to be a passive phenomenon.

The statement that injury to the endothelium or bloodcells causes an immediate local coagulation of the plasma has been confirmed by

Gutschy (32), Dietrich (21, 22a) and others, who cauterized the vessel wall or induced irritation by the application of bacterium coli.

All these observations, however, are based solely upon histological studies, to which the objection can be raised that the different fibrin stains are not specific but may stain as well hyaline and gelatinous material with a chemical constitution different from that of fibrin. Furthermore, the histological methods used by these investigators disclose only the endstage of a process and do not, therefore, justify any conclusions as to the first and the intermediate stages in this process. In addition, these investigators ignore, or at least do not take into proper consideration, the well established fact that an agglutination of certain cellular elements is generally the primary change in thrombosis, as is demonstrated in experiments in which, by the use of anticoagulants, a deposit of fibrin could be excluded. Lastly, the primary formation of a gelatinous membrane on the vessel wall could not be confirmed histologically by numerous investigators (54c, 95, 114, 43, 80, 118).

VI. The significance of inflammation in thrombosis. As inflammation represents not the change of a single factor but a combination of different processes, we can evaluate the significance of inflammation on thrombosis only by considering the relation of its various components. An aseptic local irritation of a vessel in combination with an alteration, especially a retardation or oscillation of the bloodflow, may cause a local thrombosis. Intravascular injections of large doses of microörganisms, in particular of Staphylococcus pyogenes aureus or bacillus coli, may occasionally produce thrombi. If such an injection is combined with an injury of the vessel wall, the action of the microorganisms becomes more effective (Jankowski, 42a; confirmed by Heller, 38; Dietrich 21, 22). Dietrich considers a simultaneous retardation of the bloodcurrent as an additional and essential causative factor. Perivascular application of staphylococci produces thrombi in some instances as early as after nine hours (89, 106).

However, the action of bacteria on the vessel, either following intravascular injection or injection into the surrounding tissue, goes hand in hand with an increase in the amount of proteins in the bloodplasma. Therefore, a bacterial infection, more particularly by staphylococci and colibacilli, may lead to a very extensive thrombosis, which is the stronger the more the globulin-fibrinogen-fraction predominates over the albumin fraction. Silberberg (97) infected rabbits, which had received considerable quantities of vital dyes, intravenously with

staphylococcus pyogenes aureus. He observed in these animals the occurrence of thromboendocarditis in which agglutination processes were involved. On the other hand, in cases in which either vital dye storage or bacterial infection had been applied separately, such thrombotic deposits on the heart valves were lacking. These observations demonstrate the very complex nature of the conditions underlying thrombus formation; we may conclude that a number of factors must coöperate to initiate thrombosis. McLean's (68) investigations point in the same direction: He crushed the vessel wall and applied simultaneously microörganisms to the place of injury; thrombus formation occurred at the point of injury only in those cases in which, in addition, a third factor had been active, namely, an oscillation of the bloodflow.

One is justified, therefore, in concluding that the effects of inflammation on thrombosis are not specific. Due to the different modes of action of various agents, which play part in inflammation, either an augmentation of the globulin-fibrinogen-fraction may take place, thus favoring intravascular agglutination and coagulation, or due to disintegration of tissue cells or bloodelements a liberation of tissue-or bloodcoagulins may occur, which may lead to an increased coagulability of the plasma. In still other instances, it is probable that a subsequent alteration of the protein-lipoid balance, i.e., a shift in the proportion between total proteins and total lipoids of the blood, involving a change in the proportion of coagulation-accelerating and inhibiting substances, may induce a higher coagulability of the bloodplasma or affect the agglutination process, as the case may be. To these factors there might be added, as another variable, the local interference with the smoothness of the lining of the vessel wall.

Thus, the apparently very great significance of inflammation in thrombosis is due to a summation of different factors which also, if acting singly, could cause thrombosis.

In hyperallergic inflammation, there is a slight change in the equilibrium between total lipoid and total proteins in the blood which, in certain cases, may perhaps result in a preponderance of coagulation-inducing substances, and thus thrombosis may be initiated; in other cases, the equilibrium may shift in the opposite direction, when coagulation-inhibiting substances will predominate, and thus thrombosis will be delayed. Eagle (24) has recently shown that this occurs in the case of anaphylactic shock. Therefore, as far as the hyperallergic state is concerned, the very complex mechanism underlying it may cause in some cases thrombus formation, while in others thrombosis may be

delayed by the same factors which were active also in other kinds of inflammation.

CONCLUSIONS

The experimental analysis of thrombosis leads to the conclusion that as a rule it consists of a combination of two processes, which may also occur independently of each other, namely: 1, an agglutination of the various cellular bloodelements, in particular of the thrombocytes and red cells, and 2, a coagulation of the plasma.

In lower classes of animals, agglutination processes predominate. This applies especially in vitro, but the same process can also be induced in the living animal, whereas in the more highly developed classes a combination of the two processes is seen, in which sometimes the one, at other times the other predominates, but in which, however, as a rule the coagulation process furnishes the greater quantity of the thrombus. There exists a third type of thrombosis in which solely the intravascular coagulation of the blood is concerned.

Accordingly we may classify the various kinds of thrombi as follows:

1. The agglutination thrombus which may be composed of *a*, thrombocytes; *b*, red corpuscles; *c*, white cells.

2. A combination thrombus in which agglutination and coagulation processes participate.

3. The coagulation or fibrin thrombus.

In vitro, agglutination may be promoted by slight changes of the ion-concentration in the medium in which the cells are suspended, thus causing a softening of the ectoplasm of certain cellular bloodconstituents, or by physical alterations which lead to an injury of the ectoplasmic cell layer. Agglutination in vitro is inhibited by those physical-chemical agents which cause a hardening of the ectoplasmic layer. In principle, it may be expected that these factors play a similar part in the living animal, in particular the effect of mechanical injury. The blood current has to be maintained to a certain extent to bring about agglutination. However, at the same time a slowing of the blood current may favor the formation of agglutination thrombi, especially in cases in which changes in the bloodvessel wall are concerned in the production of thrombi.

Intravascular coagulation may be due in some cases to an interaction between thrombokinase, prothrombin, calcium and fibrinogen; a direct action of tissue coagulins or blood coagulins on fibrinogen may lead to the formation of intravascular clots. The tissue coagulins are of lipoid-

protein nature; they are present in various tissues as well as in mammalian red corpuscles. They are specifically adapted to the plasma on which they act, thus resembling antibodies found in immunization. It seems that they are different from the factors present in the plasma which are concerned in the formation of fibrin ferment and which do not, as far as it is known at present, exhibit a specific adaptation to their substratum.

Anticoagulative substances which are present in the bloodserum are likewise specifically adapted to the plasma and tissue coagulins whose interaction they inhibit, and they also may be perhaps of a protein-lipoid nature.

As in the case of agglutination, the blood current has to be maintained to a certain extent for the production of intravascular coagula, but in this case again a slowing of the blood current may favor the clotting within the bloodvessels.

The close relationship between the agglutination and coagulation processes is due to the fact that mechanical factors may affect both in the same direction and, furthermore, that agglutinated cellmaterial may liberate coagulins.

Slight disturbances in the equilibrium of the proportion of the total protein and lipoid constituents of the blood, giving a preponderance to the coagulation-accelerating over the coagulation-inhibiting substances, may perhaps also cause coagulation-thrombosis.

It has not been proven that the vascular endothelia have coagulative or anticoagulative properties of a specific kind or that the formation of a fibrin membrane on the vessel wall is generally the primary factor in thrombosis. Nor is it possible to accept mechanical changes in the bloodcurrent as the primary cause of thrombosis. Hyperallergy and inflammation in general have no specific effects in inducing thrombosis, but they merely furnish conditions which also singly and independently of the inflammatory complex as such would be able to initiate thrombosis.

Thrombosis occurs when the equilibrium of the factors on which the preservation of the ectoplasmic layer of the bloodcells or the liquid state of the bloodplasma depends, is seriously interfered with. These factors may be localized in the blood itself, in the vascular endothelium or in the perivascular tissues. They may be of a mechanical, chemical or physico-chemical nature; they may be of bacterial origin and may be accompanied by inflammatory phenomena.

On the whole, the results of the more recent work on thrombosis, as

on other biological phenomena, accentuate the importance of biochemical or physico-chemical factors, in contradistinction to the tendency of scientific thought during the second half of the past century, in which special emphasis was laid on mechanical principles.

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ARTERIO-VENOUS ANASTOMOSES

ELIOT R. CLARK

Laboratory of Anatomy, University of Pennsylvania

Certain types of direct connections between arteries and veins by passages or openings greater than the diameter of a capillary, through which blood is carried without opportunity for interchange with extravascular fluids, have long been known to the clinician and pathologist. They are rare, may be properly considered pathological, and occur chiefly as a result of injury, developmental anomaly, or in vascular neoplasms. Most definite are the anastomoses resulting from stab or bullet wounds or crushing blows in which the walls of both an artery and a nearby vein are cut or broken open, permitting blood from the artery to pass directly into the vein, with the formation of a connection which may be permanent. Definite also are certain tumors, some following injury, others developing apparently spontaneously, in which there may be a number of direct anastomoses between arteries and veins, sometimes with large, coiled arteries and still wider veins. These special types of anastomoses have been extensively reviewed by Callander (1920), and Reid (1925), and will not be treated further in this review, which will concern itself principally with the question of arterio-venous connections which may occur normally.

According to the well-established views regarding the physiology of the vascular system, there would seem to be no place for normal arterio-venous anastomoses. The arteries and veins are considered chiefly as tubes conducting blood from one set of capillaries to another, while it is in the capillaries and small venules, by virtue of their thin endothelial walls, that the crucial interchange takes place between the blood and the outside tissues, spaces or fiuids, and it is this interchange which justifies the transportation portion of the system. Thus, for example, oxygenated blood would return to the lung with its content of oxygen unchanged and with no increase in its carbon dioxide content were it side-tracked from passage through the capillaries by cross-connections between arteries and veins. Furthermore, there are certain "histomechanical principles" which have been worked out chiefly by R.

Thoma (1892), according to which the vascular system is so regulated in its growth that permanent arterio-venous anastomoses would seem to be theoretically impossible, a view emphasized by Mall (1906), who, in a discussion of the operation of Thoma's "principles," in the light of his own extensive studies on the growth and distribution of blood vessels in various organs, explains how the "laws" which regulate vascular growth operate in such a way as to "prevent such a catastrophe" as the formation of persistent arterio-venous anastomoses. It is probably because of these theoretical considerations, plus the absence of direct observation on them in the living animal, that little attention has been paid to them by the physiologist; and that until quite recently their very existence has been doubted. Thus Krogh in 1922 expressed uncertainty regarding their existence, although he suggested further investigations in view of the careful morphological descriptions of H. Hoyer, Sr. (1877).

However, regardless of theoretical considerations, and in spite of predictions to the contrary, it has been possible, since the fall of 1929 to see arterio-venous anastomoses in the ears of living rabbits, both in the intact ear (Grant, 1930; Grant and Bland, 1931), and by means of specially designed double-walled transparent chambers inserted in the ear (Clark and Clark, 1930, 1934a, b), to establish certainly their existence in the normal animal, and to learn much of their behavior.

With their existence no longer a matter of doubt, we can now review briefly the results of the somewhat scattered observations which have been made on arterio-venous anastomoses in the past.

In 1844 J. Müller described direct connections between arteries and the cavernous sinuses of the penis. In 1862 Sucquet described under the name of a "circulation dérivative," direct connections about 0.1 mm. in diameter between arteries and veins in man in the skin of elbow, knee, lips, cheeks, nose, temple, eyelids, forehead, ears, fingers, toes and thenar and hypothenar eminences. In the same year Hyrtl (1862) identified as arteries emptying directly into veins structures which T. Wharton Jones (1852) had interpreted as contracting veins in the wing of the bat. J. Arnold, in 1867, described and pictured direct connections between arteries and veins in "glomeruli caudales" of dog, cat, and rabbit, which he considered homologous with the "glomerulus" or glomus coccygeum (gland of Luschka) of man. F. Berlinerblau (1875) failed to find the connections described by Sucquet, considering them artefacts. In 1877 Hoyer (Sr.) published a classical paper on "direct openings of very small arteries into vessels of venous character,"

after extensive and exhaustive injections and histological investigations. In several types of animals he found them in the ear, tip of the nose, toes of both fore and hind feet, especially at the base of the nail and in the matrix of the hoof, and in the cavernous sinuses of the sex organs. In man he found them only in the hand and foot and sex organs. He studied particularly those found in the ear of the rabbit, describing them as consisting of an arterial portion with an unusually thick muscular wall, expanding as a funnel to a much wider, thin-walled venous portion, which sometimes possessed muscle cells. Some were straight, others coiled and the inside diameters of the narrowest arterial portions measured 0.013 to 0.036 mm.

Bourceret (1885), unaware of Hoyer's studies, repeated those of Sucquet, but found in man direct connections between arteries and veins only in the pulps of the fingers, the bed of the nail and the thenar and hypothenar eminences. He described pulp arteries of 0.1 to 0.2 mm. dividing into "capillaries" 40 to 80 micra in diameter which emptied directly into veins, and found that lycopodium spores would pass through them. The name "arterio-venous anastomosis" for this type of connection appears first, so far as I have been able to determine, in 1895, used by Gerard (1895, 1896), who described anastomoses 0.4 to 3.0 mm. in diameter at the bends of the joints, and once between aorta and inferior vena cava in monkeys. Geberg (1885) and Golubew (1893) described arterio-venous anastomoses in the kidney capsule. After studying arterio-venous anastomoses in the wings of bats (1901), Grosser (1902) carried out extensive observations on the toes of various mammals, finding anastomoses present in the terminal phalanx of the toes of mouse, rat, rabbit, cat and dog, varying in inside diameter from 10 to 40 micra and in outside diameter from 55 to 90 micra. The largest were found in the wings of bats, where inside diameters measured 40 to 150 micra, outside diameters 90 to 280 micra.

Grosser studied particularly the arterio-venous anastomoses in the fingers and toes of man and has given the classical description of their histology. He found them in the beds of the nails and balls of fingers and toes, measuring 10 to 30 micra inside, 55 to 150 micra outside diameter. He gives excellent reconstructions of the coils, or "snarls" made by the twisted arterial portion, and describes the greatly thickened musculature as having, in addition to the usual circular arrangement found on arteries and arterioles, an inner longitudinal layer and an outer oblique layer, rich in nuclei, although both the longitudinal and oblique layers might be missing. He found elastic fibers in some

cases, but an absence of a definite elastica interna, and emphasized the richness of their nerve supply.

Vastarini-Cresi (1902) also gave an excellent description of arterio-venous anastomoses in the fingers and toes of mammals, emphasizing three portions—the arterial, the funnel-shaped venous, and the thick-walled intermediate portion in which, like Grosser, he found inner longitudinal and outer oblique layers. He suggested that the thickened portions may act as sphincters.

Walker (1904) concluded that the cells surrounding the endothelial tubes, in the glomus coccygeum of man, were true epithelial cells and argued that these bodies should be grouped with the ductless glands.

In 1907 Schumacher studied histologically the glomus coccygeum of man and the glomeruli caudales of other mammals. Like Arnold, he found arterio-venous anastomoses conspicuous in them, and emphasized the "epithelioid" nature of the outer layers of the thickened portion, which he considered to be probably modified muscle cells. He did not find them expecially rich in nerves. The same year Stoerk (1907) failed to find chromaffin tissue in the glomus coccygeum, as well as any histogenic relation to the sympathetic system. In a later paper Schumacher (1915) stressed the "epithelioid" modification of the muscle in arterio-venous anastomoses in the toes of birds.

In 1912 Nussbaum described arterio-venous anastomoses in the subpericardial vessels. He considered them, however, as merely widened capillaries and not anastomoses in the sense of Hoyer and Grosser, since their wall consisted of endothelium alone, with no muscle cells.

Miller and Godfrey (1917) described the finding, in a cat, of a total of 23 unusually large anastomoses (0.2 mm. to more than a millimeter in diameter) between some of the main arteries and veins chiefly in the posterior abdominal region and the posterior extremities.

Clara (1927) studied extensively the histology of the walls of arterio-venous anastomoses in birds and mammals, and found that some, particularly the coiled ones, had "epithelioid" modifications of muscle cells, while straight ones did not. He also stresses the absence of an elastica interna.

In Testut's *Anatomy* (1929) there is an account of a search for arterio-venous anastomoses in the pia mater, where their presence had been alternately affirmed and denied, with the conclusion that they are present there, but that they are so rare that, as far as this region is concerned, they should be considered as "simple morphological accidents."

Brown (1937) describes, very briefly, arterio-venous anastomoses in the dog's tongue, emphasizing the presence of a very rich nerve supply consisting of thin, unmyelinated fibers with terminations in the "media," and thick myelinated fibers with terminations mainly located in the "adventitia."

The studies thus far reviewed would seem to have established the fact that definite anastomoses between arteries and veins, larger than capillaries, but rarely exceeding 50 micra inside diameter, are normally present in birds and in mammals including man, at least in certain definite localities. The most definite places in which they have been repeatedly found are: in birds—at the base of the claws; in animals other than man—the penis,—in the form of helicine arteries emptying directly into the cavernous sinuses; the terminal phalanges of both fore and hind feet of both claw-bearing and hoof-bearing animals,—in some cases only at the base of the claw or hoof, in others in the toe pads; in the glomeruli caudales; in the ears; and in the nose or snout. In man arterio-venous anastomoses have been found definitely and consistently only in the penis, the balls and beds of the nails of fingers and toes (perhaps also the volar sides of the fingers and palms of the hands) and in the glomus cocygeum. While they have been described sporadically in other localities by various investigators, and while it is possible that they may have a wider distribution, the question as to their normal occurrence in other places than those just mentioned awaits further verification.

It is rather remarkable that, until 1924, these interesting structures remained of little more than academic interest. With the exception of those in the sexual organs they were practically ignored by both physiologist and pathologist. Whatever speculations there were regarding their possible function, were made by the investigators who described them, representing merely the usual attempt to justify the existence of a newly found structure.

During the past 13 years, however, there have been three groups of investigators who have brought arterio-venous anastomoses to life both for the physiologist and for the pathologist and clinician.

In 1924, P. Masson published the first of a brilliant series of studies on the arterio-venous anastomoses particularly of the fingers and toes in man in relation to certain painful tumors in which they are apparently involved (Masson, 1924, 1927, 1935, 1937). He thus linked the *normally present* arterio-venous anastomoses for the first time with a pathological condition. His starting-point was the study of 3 small

sub-ungual tumors. In looking for nearby structures from which these tumors may have arisen, he saw scattered structures of similar morphology, which he first mistook for other small tumors, but which he later found to be the arterio-venous anastomoses described years ago by Hoyer, Grosser and others. This led him to the most exhaustive and penetrating study which had yet been made of their morphology and, with his co-worker Popoff (1934, 1935) to a study of their variations in different ages and their behavior in pathological conditions such as inflammation, arteriosclerotic gangrene, diabetic gangrene, and thromboangiitis obliterans.

From the start, Masson was dissatisfied with the name "arterio-venous anastomosis" because of the confusion with the traumatic and other unusual connections which have received the same name. Since those in the finger often occur in the form of a group which makes a sort of ball, or skein, and since they have a morphology similar to those found in the glomus coccygeum—the one other place in man beside the hands and feet and sexual organs in which they are certainly present normally—he adopted the term "glomus." Later, in view of their great richness in nerves, he enlarged the name to "glomus neuro-vasculaires" (Masson, 1937). Tumors involving them he termed "glomus tumors" or "glomic tumors" ("angio-myo-neuromes artériels").

The introduction of this new name has had such a remarkable effect in lifting the arterio-venous anastomosis from its position as a neglected and almost forgotten structure to one of prominence, at least among pathologists and clinicians, that Masson in 1937 lists in addition to his own, no less than 34 titles of articles on "glomus tumors" published since 1924.

While the term "glomus" has undoubtedly served a useful purpose in attracting attention to the tufts of arterio-venous anastomoses in fingers and toes, it seems wiser to me to use the more time-honored name to cover all direct connections between arteries and veins. Many typical normal anastomoses occur singly rather than in tufts, and it would still be necessary to use for them the term arterio-venous anastomosis. Moreover, the term "glomus" is in danger of being somewhat over-worked, and to cause confusion if used for structures which have nothing in common. Thus Boyd (1937), in order to avoid confusion, prefers the term "body" for the "carotid body," which has been called, among other names the "glomus caroticum," and he would use the term "aortic bodies" rather than "glomus aorticum" (Nonidez, 1935)

to designate the paraganglia which are, at the present time, of such great interest to anatomists and physiologists. Masson (1937, p. 4) himself protests the use of "glomus" for the carotid and aortic "paraganglia," which appear more and more definitely to have little if anything in common with the "glomus coccygeum." Popoff's (1934) name "Sucquet-Hoyer canals" for arterio-venous anastomoses seems quite superfluous.

It is possible to give but a brief summary of the chief results of Masson's (1937) comprehensive account of the arterio-venous anastomoses found in the human fingers, some of which are confirmatory of previous studies.

They are located with diminishing frequency in the bed of the nail, palmar digital pulp, lateral digital pulp of the terminal phalanx, digital pulp of the middle phalanx, and digital pulp of the proximal phalanx, a relative distribution also found by Grant and Bland (1931) and Popoff (1934). In the pulp region they are found chiefly intradermally but to some extent in the subcutaneous region. They may occur as single or double connections or as "bouquets" of multiple anastomoses—in places the "bouquets" being almost contiguous. The thick-walled arterial segments after a straight or winding course empty directly into wide, thin-walled veins, the whole making a sort of ball or "glomus."

The arterial segments are characterized by the absence of an elastica interna, and by a thick wall, made up of several layers, of which the inner cells are typical smooth muscle cells, while the outer show an "epithelioid" modification. Careful study has convinced Masson that the latter are probably modified smooth muscle cells. Like others, he finds both longitudinal and oblique fibers among them, but in addition he considers that the cells of the outer layer form a syncytium, anastomosing in all directions, and with scanty fibrils passing from cell to cell—an arrangement which he compares with the myocardium. Surrounding the arterial segments Masson describes and illustrates profusely an extraordinarily rich "manchon" (muff) of nerves, which consists of both fine, non-medullated fibers extending out from the network on arteries and veins, and of coarser, irregular, nodulated, non-medullated fibers which may be traced to medullated nerves of the dermic plexus. The latter could be followed, on occasion, to irregular endings among the epithelioid cells.

Masson finds anastomoses, identifiable by the thickness of wall and epithelioid cells, in fetuses of 4th and 5th months, but without the extra rich nerve supply; and he finds them less numerous in old age than

in adults or children. The behavior of these structures in arteriosclerosis and in diabetes and thromboangiitis obliterans has been studied by Popoff (1934). The small, benign, but often very painful "glomic tumors" are described by Masson in great detail. They present all the characteristics, merely exaggerated in degree, of the normal "glomus vasculaires," namely, enlarged and lengthened arterial segments, conspicuous "epithelioid" cells, and great richness in nerves. While not especially common, they are likewise not rarities, Masson having encountered 27 personally, and received 20 from colleagues.

Masson has summarized his 13-year studies in a monograph (1937) which is remarkable for the clarity and brilliance of its description and analysis, and for the originality and charm of its language—to none of which has the above meagre review done justice.

While Masson has vivified the normal arterio-venous anastomosis particularly for the pathologist and the clinician, this long-neglected structure has been elevated during the past 7 years to a position of importance for the physiologist and anatomist as a result of the development of methods which have made it possible to observe arterio-venous anastomoses in the living animal, to establish without question their existence, to discover many of the details of their behavior both normally and in response to heat and cold and to drugs, and to find out some of the factors which are responsible for their formation, maintenance and disappearance.

In 1930 Grant, from Sir Thomas Lewis' laboratory, published the results of studies made on arterio-venous anastomoses as seen in the intact ears of living albino rabbits. He found it possible, after using a depilatory and while employing sodium luminal or urethane anesthesia, to see occasional arterio-venous anastomoses in the living animal by means of the pulsation at their point of entry into the vein when they were dilated, while in fixed and stained preparations he demonstrated that such pulsation points were true arterio-venous anastomoses. He saw "oscillations" in caliber of arterio-venous anastomoses during a period of an hour and a half in which no changes in the arteries were seen.

When the body temperature of the rabbit was raised, Grant noted that the anastomoses dilated, and that, in cooling, they closed. When the ear alone was heated he found that at 35°C. the arteries dilated, but that the arterio-venous anastomoses dilated at 40°C. On cooling the ear below 15°C. he found that arterio-venous anastomoses dilated and all other vessels constricted, although with prolonged cooling the ar-

teries also relaxed. Upon reheating the anastomoses constricted before the arteries. Local mechanical stimulation, acetyl-cholin and histamin caused dilatation, while adrenalin and faradic stimulation of the sympathetic produced constriction of arterio-venous anastomoses.

He concludes that arterio-venous anastomoses are important in regulating body temperature—that they aid in heat loss by allowing an enormous blood flow through the ear, when the animal is overheated, and the reverse when cooled. He considers that the ear is protected against over-cooling by their dilatation at very low temperatures.

Grant and Bland (1931) studied the arterio-venous anastomoses in the toes of birds and in the fingers and toes of man. With the aid of thermocouples, the temperature of the skin of the fingers at several different places was recorded, while the tip of the finger was held in iced-water for a time and then warmed. Simultaneous observations were also made on dermal capillary loops. The evidence, though indirect, was conclusive that the arterio-venous anastomoses of the fingers are active in contraction and dilatation and that they play an important rôle in the temperature changes of the skin of the fingers, and in the temperature and content of the blood in the veins of the forearm.

Similarly Lewis and Pickering (1931), by the simple expedient of placing a rubber band around the proximal phalanx of a finger, thus shutting off a venous return which would have contained blood side-tracked through the arterio-venous anastomoses of the finger, found greatly retarded temperature changes at the base of the finger as compared with the base of a finger with unimpeded circulation.

Grant, Bland and Camp (1932) later carried out temperature recording experiments on the rabbit's ear, similar to those made on the fingers, using partial immersion of the ear in cold water, and, in some cases measured the diameters of sample arteries and arterio-venous anastomoses. They found that the latter took part, along with the arteries, in the various constrictions and dilatations producing temperature changes in parts of the ear, which were similar, in general, to the types of temperature changes recorded in the human fingers. These studies increased the probability that the temperature changes in the fingers are appreciably modified by the arterio-venous anastomoses.

While the observations made in Sir Thomas Lewis' laboratory, on the occasional arterio-venous anastomosis which may be seen through the skin of the intact rabbit's ear, have produced data of great value regarding certain details of their behavior, a much more intimate knowl-

edge of arterio-venous anastomoses in the living animal has been made possible by the development of various types of transparent double-walled windows during the past 15 years, which, permanently installed in the ears of rabbits, have enabled the observer to see with highest microscopic magnifications the most minute details of arteries, capillaries, veins and arterio-venous anastomoses. These studies have served to establish the existence of the latter beyond possibility of doubt; to discover many interesting facts regarding their behavior both in normal and experimental conditions; to watch their manner of formation and to work out some of the factors which regulate their growth, maintenance and retrogression. The chambers are of two types. In the original type, the details of which were developed by Sandison (1924), from which was evolved the "round-table" chamber (Clark, Kirby-Smith, Rex, R. G. Williams, 1930), a space is left into which there occurs a growth of new vessels and other tissues. In a second type (Clark et al., 1930), the original subcutaneous tissue of a portion of the ear, after removal of the skin of one or both sides and of the cartilage, is permanently enclosed within a double-walled chamber. In the latter type it is possible to study the original arterio-venous anastomoses, many of them with nerve supply intact, and in the former, the new-formation of arterio-venous anastomoses and their behavior. Observations are made on the unanesthetized rabbit, lying on its back, held in such a way that it apparently suffers no discomfort, and with the ear horizontal. With special holders the ear may be kept sufficiently still for even oil immersion camera lucida drawing, photomicrographs and motion-pictures, including the "stop-motion" type. Continuous observations may be made of seven hours' or more duration, repeated daily for weeks or months (several chambers have lasted for two years), without the slightest impairment of the general condition or disposition of the rabbit.

Some of the chief results of this study are the following (Clark and Clark, 1930, 1932, 1934a, b). There are many arterio-venous anastomoses normally present in the rabbit's ear, through which a relatively enormous amount of blood may pass into veins without traversing the capillaries, and with no noticeable change in the brick-red color of arterial blood. In an area of approximately 1.6 sq. cm. of the subcutaneous tissue dorsal to the cartilage, counts in many rabbits have ranged from 25 to 50. In general, active, lively rabbits were found to have a lively circulation and a larger number of arterio-venous anastomoses than more phlegmatic rabbits with less active circulation. When

a daily census was made of all the anastomoses present in a series of chambers over periods of 1 to 3 months it was found that most of them persisted. A few subsided while a small number of new ones appeared. Thus, for example, in one chamber 41 arterio-venous anastomoses were counted 39 days after installation, of which 38 had persisted for 33 days of observation, 2 of the original 40 having subsided and 3 new ones developed.

The arterio-venous anastomoses showed in the main the characteristics described by Hoyer and others in fixed preparations. Some were straight, others coiled. The large majority showed the 3 portions, the usual arterial, the intermediate portion with a thicker muscular layer and a narrower lumen, and the thin-walled, funnel-shaped venous portion. The intermediate portion showed the most active contractility, the venous portion being non-contractile. In some, however, the muscular arterial wall extended from artery to vein without appreciable increase in thickness or contractility. The majority arose from smaller arteries but a certain number arose from larger arteries, or from arterioles, while others formed the termination of an artery or arteriole, and clusters of three or more from the same artery were frequently seen. While the venous portion was thin-walled and non-contractile, it was found that larger veins which received the outlet of many arterio-venous anastomoses often had substantial muscle walls, and showed definite contractility. The size, or inside diameters, measured at the thick-walled, intermediate portion, varied greatly, not only in different arterio-venous anastomoses, but in the same one in different states of contraction or dilatation on the same day, and on different days. The greater number showed inside diameters at their narrowest point during dilatation of 20 to 40 micra. However, typical anastomoses were observed measuring no more than 5 micra, and ones measuring over 40 micra were not infrequent. The largest one which was seen measured 63.3 micra. When it is recalled that, according to Poiseuille's laws, the amount of flow through capillary tubes varies with the 4th power of the diameter, the relative amount of blood which may pass through these anastomoses is enormous. Thus 256 times as much blood should pass through an anastomosis 40 micra in diameter as through a capillary 10 micra in diameter, of the same length, and this is probably an underestimate of the actual excess.

In their behavior arterio-venous anastomoses have much in common with arteries. It has long been known that the arteries of the rabbit's ear are active, as regards contraction and dilatation, but the extent and

variety of their activity had not been appreciated until they were studied in the transparent chambers. There it has been found (Clark and Clark, 1932) that most of the arteries and arterioles of the ear undergo periodic contractions at different rates, most commonly about two times per minute, even when a conditioned rabbit is lying quietly, and protected from any auditory or other disturbances. However, the rate of periodic contractions may vary in different arteries and arterioles—rates of 12 per minute in certain arterioles having been observed. This irregular contraction plays an important rôle in causing extraordinary variations, including reversals of flow, in arterial anastomoses and in veins. Furthermore, following slight disturbances to the rabbit such as noises and proddings of the animal, both of which produce muscular jerks, the arteries and arterioles react promptly by a contraction which persists for periods of time varying from 10 to 20 seconds, when they again relax. Moderate cooling of the rabbit or of the ear alone causes narrowing of arteries, while moderate heating of the rabbit or the ear results in dilatation. The periodic contractions continue during both moderate heating and cooling, but are abolished by excessive heating or cooling, as well as by sodium amytal anesthesia and during sleep.

In general it may be said that the arterio-venous anastomoses behave in much the same way as arteries and arterioles, the chief difference so far discovered being one of degree, for they are decidedly more active even than arteries and arterioles, and individual arterio-venous anastomoses show a greater tendency to independent action. Thus, arterio-venous anastomoses contract and dilate spontaneously and periodically, as do the arteries, but each may have a rhythm independent of other nearby anastomoses, as well as of the artery from which it arises—and usually at a faster rate than the arteries. On the other hand, certain of them may remain persistently contracted while others are showing periodic changes. Like arteries, the arterio-venous anastomoses contract, when the rabbit gives a slight struggle following auditory or physical disturbance, differing only in that they react more quickly and speedily. Thus the lumen of an arterio-venous anastomosis 20 micra in diameter is obliterated by contraction in about ½ second, while an arteriole of the same size takes about 1½ second. So rapid is the action of the arterio-venous anastomosis that it resembles a rapidly acting stop-cock. Similarly arterio-venous anastomoses are wider in response to local or general heating, and narrower in response to cooling, although there are usually certain exceptions in both cases. Periodic

contractions continue at slightly higher temperatures than in arteries, but are eventually abolished. Near regions of local infection arterio-venous anastomoses, like arteries, remain persistently wider than normal, and usually cease contracting periodically.

When the nerves to arterio-venous anastomoses are injured, they remain continuously dilated for 10 to 14 days, after which the muscle acquires tonicity and the lumen is thereafter maintained at a narrow caliber. During such a period of prolonged dilatation of arterio-venous anastomoses, arterioles and arteries, with uninjured nerve supply, may remain quite narrow, with very little or no flow through the capillaries. In all those conditions, on the other hand, in which both arteries and arterio-venous anastomoses are equally dilated, blood continues to flow through capillaries in spite of the enormous amount of side-tracking of blood. However, there may be an exaggerated amount of plasma-skimming in arterioles beyond the last arterio-venous anastomoses, owing to the diversion of the bulk of the blood.

Regarding definite clues as to factors which may play a part in regulating the size and persistence of arterio-venous anastomoses, it was considered suggestive that in a rabbit with a chamber in each ear, in which the tissue in one ear was kept unusually free from injuries, sudden temperature changes and infection, while the other was subjected to such influences, over a period of several months, there were nearly twice as many arterio-venous anastomoses in the latter at the end of the period as in the former. Further evidence in this direction was derived from the study of newly-formed arterio-venous anastomoses.

That new arterio-venous anstomoses may form in the adult rabbit has been abundantly proven by the direct observation of this phenomenon hundreds of times in the other type of transparent chamber, in which all the vessels present are newly-formed, or regenerated. The first suspicious one was a small one described by Sandison (1928). Later, in the "round-table" chamber (Clark and Clark, 1934b) abundant opportunity has been afforded to study their formation, activity, life-history, and some of the factors which seem to be responsible for their formation. The chief results of these studies have been summarized as follows (Clark and Clark, 1934b, pp. 453, 454).

1. Development of new arterio-venous anastomoses from newly-formed blood-vessels which have grown into transparent "round table" chambers inserted in the ears of rabbits has been observed repeatedly in the living animal. The basis for their formation is universally present in the primitive arterial-venous capillary connections.

2. Microscopic observations show that new arterio-venous anastomoses of characteristic shape form within a few days from small vessels already present in the vascular plexus, by enlargement of the lumen, straightening of their course and thickening of their walls through the addition of extra-endothelial cells resembling the smooth muscle cells on the arterioles. The exact origin of these cells has not yet been determined.

3. The size of newly-formed arterio-venous anastomoses during dilatation showed extremes of 5.6 and 60 micra (inside diameter), the majority ranging from 20 to 35 micra. Measurements of capillaries in the same areas showed a usual range of 4.25 to 12.6 micra in circulating vessels.

4. Stimuli which cause a sudden increase in circulation followed by 2 or 3 days of increased blood flow may lead to the formation of numerous arterio-venous anastomoses in stable vascular networks. The circulatory conditions which result in the formation of new arterio-venous anastomoses have been produced experimentally.

5. Most of the newly formed arterio-venous anastomoses are temporary. With return of quieter circulation in the vascular area, they diminish in size, lose some of their extra-endothelial cells and either become inconspicuous portions of the capillary network, or retract and disappear.

6. Among the numerous arterio-venous anastomoses found from time to time in the transparent chambers some have been observed to persist as relatively permanent connections (for a month or more). Of these "permanent" arterio-venous anastomoses some remain non-contractile, while others have been observed to develop definite active contractility. The contractile arterio-venous anastomoses show essentially the same behavior as do the arterio-venous anastomoses with intact nerve supply present in "preformed tissue" chambers and, like them, appear to be under both central and local control. There is evidence that the acquisition of active contractility by certain of the newly-formed arterio-venous anastomoses is associated with regeneration of nerves.

7. Some permanent arterio-venous anastomoses were seen to grow small and disappear. Contributing factors were, a, lessening of blood flow; b, formation of new capillaries supplied by the artery beyond the site of origin of the arterio-venous anastomosis; c, persistent maintenance of a completely contracted condition in the arterio-venous anastomosis. Similar disappearance of arterioles has been observed, associated with the same contributing factors.

In this review there has been no mention of the various speculations which have been proposed regarding the "purpose" of arterio-venous anastomoses, nor will there be. It is my opinion that already altogether too many erroneous notions have crept into physiology as a result of unbased teleological explanations. Far healthier, I believe, is the attitude that definite physical and chemical factors will be found to account for structure.

Considered from this standpoint, there is much information which has been already gained regarding arterio-venous anastomoses which seems to aid in explaining their existence, their distribution and their

comings and goings, and which may form the basis for an understanding of the development of anastomoses in places where they are not customarily found, and perhaps of some of the types of pathological arterio-venous anastomoses.

It is significant that the most definitely established locations in which they occur normally in great numbers are parts of animals which are most frequently subjected to various types of irritations—mechanical and thermal—which are known to produce more or less prolonged local dilatations of arteries and arterioles. These are the tissue at the base of the claws of birds and of the nails, claws or hooves of mammals; the tissue of the finger and toe pads of many mammals; the tissue at the base of the beak of birds, and the nose or snout; and the tips of the tails of certain mammals. All these places are subjected frequently to mechanical insults; while the ears of certain animals, such as the rabbit, are subjected frequently to both thermal stimuli and mechanical insults. These are the very conditions which have been found by observation and experiment in the rabbit's ear to bring about successive crops of new arterio-venous anastomoses, as well as an increase in the size and number of those already there.

An interesting question is raised in Mall's (1906) discussion of Thoma's "histomechanical principles" of blood-vessel growth, which seem to him to exclude the possibility of the persistence at the same time of arterio-venous anastomoses and capillaries. The reconciling factor, overlooked by Mall, apparently lies in the contractility of the arterio-venous anastomoses. If they were not contractile, but remained dilated continuously, the fate predicted for capillaries—elimination—would probably occur, as a result of the shunting of the blood. Furthermore, if the dilatation with full flow of blood continued without remission, the arterio-venous anastomoses would increase in size and number. This may be a partial explanation for the development of certain types of arterio-venous anastomotic tumors—though it leaves unexplained the cause of the persistent dilatation. But in the normal behavior of the peripheral vessels, as observed in the living, arteries, arterioles and arterio-venous anastomoses are partially or wholly contracted a considerable percentage of the time. The partially contracted condition favors circulation through the arterioles which lead to the capillaries, since the arterio-venous anastomoses, having a thicker muscle wall, are contracted to a narrower caliber than the arterioles of corresponding size. Furthermore, alternation of circulation between the two is favored by the fact that their times of

contraction and dilatation frequently do not coincide. Again, while both may remain completely closed for considerable periods, arterio-venous anastomoses manifest this tendency to a greater extent than do the arterioles, again favoring the flow through the capillaries. It is apparently this alternation of the flow, produced by irregularities of contraction and dilatation, which makes possible the survival of arterio-venous anastomoses and capillaries in the same region. And, if this is so, as a corollary one would expect to find an absence of arterio-venous anastomoses in organs in which there is little or no irregular contracting and dilating of arteries. This expectation has been borne out in two regions in the rabbit—the mesentery (Zintel, 1936) and the pia mater (Wentsler, 1936), where an absence of irregular contractility has been accompanied by absence of arterio-venous anastomoses.

That arterio-venous anastomoses are more richly supplied with nerves than corresponding arterioles seems well-established by the observations of Masson and others. The possible function of this excessive nerve supply has been discussed by Masson, but its full significance awaits further study. The same may be said of the so-called "epithelioid" modification of the outer cells of the arterio-venous anastomoses of the human fingers and toes.

The coiled and twisted shapes of many arterio-venous anastomoses may be due to their lack of an elastica interna.

Regarding the function of arterio-venous anastomoses, the most one can say definitely is that, when dilated, they permit a large amount of blood to pass from artery to vein without passing through the capillaries. They undoubtedly play a part in the surface heating or cooling of the blood in the human hand and forearm and in the rabbit's ear, since they must allow a greater total flow through the exposed veins than would be possible without them. The same may apply to the ones very recently described by Brown (1937) in the tongue of the dog. Yet one cannot accept the view of Grant that the opening of arterio-venous anastomoses at very low temperatures suffices to protect the part itself from becoming too cold, since it is common observation that fingers and toes may freeze if subjected too long to very low temperatures. It is difficult to assign a similar function to those which have been described in the toes of birds and animals, in the tail, the nose and in the glomus coccygeum.

It is not impossible that, in such locations, they may represent merely an unfortunate type of reaction of the blood vessel to special circulatory conditions, and that no useful function whatsoever may be served by them. Certainly if enough of them were present and remained

open, there would result an increase in the amount of the blood and in the size of the heart—a condition which has been found in the case of large, pathological arterio-venous anastomotic tumors (cf. Callander, 1920).

With the definite establishment of the normal existence of arterio-venous anastomoses in certain regions, and with only a beginning made regarding their behavior in normal and pathological conditions, many problems press for solution. Concerning distribution, are they present in other locations normally? Are they present in other regions in some individuals and not in others? What is the range of variations according to age, sex, and type?

Regarding the extent to which they remain open, it should be possible to secure data by extending the temperature studies of Lewis and Grant, and by estimating the oxygen content of blood drawn from the veins of the back of the hand or arm, under a variety of conditions and in different types of individuals. The amount of dilatation or contraction of the arterio-venous anastomoses of the fingers should be taken into account in analysing blood samples from the arm veins. It should be possible to produce excessive numbers of them experimentally perhaps to the extent of causing the formation of tumors, and then to investigate the feasibility of bringing about their reduction. Conditions in which enlarged veins occur, such as certain cases of hemorrhoids, should be studied with a possibility that they may be associated with abnormal arterio-venous anastomoses.

Many other questions and lines of investigation will suggest themselves, for normally present arterio-venous anastomoses have at last been raised to a place of importance and can no longer be ignored by the morphologist, the physiologist or the pathologist.

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UNDERNUTRITION AND CARBOHYDRATE METABOLISM

WILLIAM H. CHAMBERS

Department of Physiology, Cornell University Medical College, New York

Undernutrition is used in a broad sense in this paper to include complete inanition as well as the dietary deficiencies. Fasting has been recognized as a factor in carbohydrate metabolism since 1873, but the greatest expansion of the entire field has occurred in the past 15 years. During this time the endocrine control of carbohydrate metabolism has been developed and now occupies a position of major importance. An attempt to correlate the studies of undernutrition and the endocrine research has led to the following generalizations concerning carbohydrate utilization.

The maximum utilization of administered glucose is found in the normal individual when carbohydrate has been abundantly supplied in the diet. The minimum effect is obtained in the completely depancreatized animal which excretes all of the ingested sugar in the urine. Between these extremes is a series of less sharply defined intermediate stages. As the amount of carbohydrate in the diet is diminished the glucose tolerance test indicates a decreased utilization. During complete inanition there is a progressive deprivation of the carbohydrate stores in the body and an increasingly larger fraction of the test meal is lost by excretion as the fast continues. A more drastic and exaggerated condition is seen in the animal depleted by phlorhizin glycosuria. Approaching pancreatic diabetes at the lower end of the scale are the various stages of diabetes mellitus and the endocrine disturbances involving the pituitary and the adrenal. Of the many factors exerting a controlling influence on the different degrees of carbohydrate utilization as outlined, the importance of two is apparent—the amount of carbohydrate available to the cells and the endocrine control. The former seems to predominate at the beginning of the series but is superseded by the latter toward the minimum end. The literature presented in this review will give the evidence supporting these generalizations, with particular emphasis on the nutritional condition with respect to carbohydrate.

The conception of carbohydrate metabolism outlined above is based on the measurement of the body's ability to utilize carbohydrate, as determined by its reactions for several hours after the administration of pure glucose. When a glucose test meal is ingested sufficient in amount to maintain the maximum rate of absorption from the intestines for several hours, the total heat production of the body indicates that in the normal well-fed organism only about one-quarter to one-third of the absorbed sugar can be oxidized to CO_2 and H_2O. The major portion of the remainder is probably stored as glycogen, but conversion into fat, glycolysis to lactic acid, transformation into hexosephosphate, glycerophosphate, glucosamine, or other compounds may also occur. These reactions may be observed in several ways. The method most extensively used for the study of carbohydrate metabolism has been the glucose tolerance test in which the rise and fall in blood sugar concentration are determined for several hours after the administration of sugar. This test is most useful in showing small differences in utilization. It should be remembered that the blood sugar changes reflect the rate but not the nature of glucose utilization. If the impairment is large enough, information as to the total amount utilized may be obtained from the quantitative collection of the fraction excreted by the kidney. The most direct evidence for oxidation comes from carefully conducted determinations of the respiratory metabolism sufficient in duration to yield combustion quotients when interfering changes in the body are adequately controlled (Richardson, 1929). Tissue analyses yield data concerning glycogen deposition and other methods of disposal. All of these reactions must be considered in establishing a complete picture of carbohydrate utilization. The terms "utilization" and "oxidation" have been used synonymously in some of the recent literature. This is obviously inexact in short experiments.

Historically, research in this field started with a dissertation in 1873 on arsenic poisoning and liver glycogen by Lehmann in which he noted that control rabbits after fasting for 2 or 3 days exhibited a glycosuria upon the intravenous injection of sugar. About the same time Claude Bernard (1877) referred in his chapter on artificial diabetes to an observation that traces of sugar appeared in the urine of fasted dogs after starch ingestion. The pioneer quantitative study of the impaired assimilation of glucose in fasted or undernourished animals was made in 1890 by Hofmeister, who named the condition "hunger diabetes." He found that the increased sugar excretion of dogs in this condition was related to the amount of carbohydrate fed and was not associated

with a more rapid absorption of glucose from the intestine. These early studies concerning the development of glycosuria were fortified by Johansson's (1909) discovery that the normal rise in CO_2 production after dextrose was absent in fasted individuals.

The advent of micro methods for blood sugar determinations gave a new impetus to this means of studying glucose metabolism. Bang (1913) discovered that the rise in blood sugar following the administration of glucose was more marked in fasted than in well-fed rabbits, an observation which was confirmed by Böe (1913) and by Elias and Kolb (1913). The converse was likewise demonstrated by Bang, i.e., a second dose of glucose produced less hyperglycemia than the initial one. The latter phenomenon, the augmentation of utilization by repeated doses, was demonstrated in normal and mildly diabetic humans by Hamman and Hirschman in 1919 and extensively studied by Staub and by Traugott in 1921 and 1922. The work cited above opened the way for later research which has contributed knowledge of fundamental value to the present concepts of carbohydrate metabolism and to the dietary treatment of diabetic patients.

DIET. The inhibitory effect of a low carbohydrate diet on the assimilation of sugar, reported by Staub and by Kageura in 1922, was soon confirmed by many investigators. In the four patients observed by Staub (1922b) the tolerance for 20 grams of glucose was tested after 2 days of a diet composed of protein and fat and compared with a diet high in carbohydrate. The hyperglycemia after the test meal following the protein-fat diet was definitely higher in each case. Similar results on man and in a series of experiments with dogs were published by Kageura (1922a, b). The idea was supported and amplified by the studies of glycosuria made by Southwood (1923) and by Greenwald, Gross and Samet (1924) and by the extensive work of Adlersberg and Porges (1926, 1929) on normal subjects and diabetic patients.

The duration of the diet seems to be a variable of major importance only at the beginning of the dietary experiments, whereas in complete inanition the changes become progressively greater. There has been some disagreement as to how quickly the effect of a change in diet could be demonstrated. A single meal was reported by Staub (1922b) to have no influence on the glucose tolerance, and likewise Kohn, Fries, and Felshin (1927) found normal reactions in children on the first day of a ketogenic régime. However, the data of Adlersberg and Porges (1926) indicate an appreciable difference in some cases. Holtz (1931) also has noted definite changes in 20 kgm. dogs after very large single

meals of 500 cc. of oil, 600 grams of sugar or 3000 grams of meat. The average area of the blood sugar tolerance curves was 370 mgm. per cent hours after carbohydrate, 420 after protein, and 1450 after fat. The maximum effect of a particular diet seems to be reached by the 2nd to 4th day and a longer period on the same diet (19 days to 1 year) does not appreciably alter the condition (Kageura, 1922; Malmros, 1928; Tolstoi, 1929; Fanconi, 1931; Clark and Greene, 1935; see also table 2).

Protein. The rôle of protein in the diet became a subject of controversy involving the question of whether or not protein contributes to the carbohydrate portion of the diet in the normal as in the diabetic organism. It was thought by Kageura to be no more effective than fat in promoting carbohydrate utilization, but an objection to this finding was raised by Greenwald, Gross, and Samet (1924) on the basis that over 50 per cent of the calories were supplied by fat in the high protein diet used by the former author. The latter investigators analyzed by several methods the daily sugar excretion of a human subject and of two dogs when various combinations of protein, fat, and carbohydrate were taken. The addition of 170 grams of glucose to a diet of 600 grams of beef heart alone caused no significant change in the amount of sugar excreted by the dog but some increase when the previous diet consisted of 1200 grams of kidney. When fat was substituted for a part of the protein, the same amount of glucose added to a ration of 160 grams of beef heart plus 110 grams of lard raised the glycosuria from 0.43 gram per day to 1.07 grams per day, thus showing a diminished utilization of sugar. With the glucose tolerance test the protein diet is placed in an intermediate position between that of fat and of carbohydrate, according to the data of Sweeney (1927). The test meal of 1.75 grams of glucose per kgm. of body weight was taken by groups of students after 2 days of the special diet. The average maximum blood sugars were as follows: for the predominantly starchy diet, 118 mgm. per cent; for the protein (lean meat and egg white), 167 mgm. per cent; and after fat, 206 mgm. per cent. A normal glucose tolerance was found by Heinbecker (1928) in Eskimos on their native diet, which was estimated to contain 280 grams of protein, 54 grams of carbohydrate, and 135 grams of fat, essentially a high protein ration, although from a similar study Rabinowitch and Smith (1936) conclude that carbohydrate utilization is somewhat impaired in the Eskimo.

With the more drastic test of a constant intravenous infusion at the rate of 2 grams of glucose per kgm. of body weight per hour Wierzuchowski (1931b) also placed protein in an intermediate position between

carbohydrate and fat. Hourly changes in blood sugar concentration and urinary excretion were determined during 10 hours of injection. For a period of one week before the test the ration contained either lean meat exclusively, 90 per cent of fat, 90 per cent of starch, or 90 per cent of sucrose (1931a). After the high carbohydrate diets 9 per cent of the injected glucose was excreted, as compared to 25 per cent after meat and 33 per cent following the fat diet.

The oxidation of ingested glucose is but slightly impaired by a prolonged meat diet according to the respiratory metabolism determinations made in this laboratory (Chambers and Chandler, unpublished data). Four dogs were fed for 2 to 3 weeks solely on lean beef heart which contained only 30 mgm. of glycogen per 100 grams of tissue. The amount (600 to 900 grams daily) was sufficient to maintain the body weight. In each case the basal respiratory quotient was 0.77 and after the administration of 50 grams of glucose the quotients

TABLE 1

Diet: Protein (*per cent*)	24.2	51.0	74.4	15.9	13.8
Fat (*per cent*)	21.0	22.6	19.7	55.0	80.1
Carbohydrate (*per cent*)	54.8	26.4	5.9	29.1	6.1
Blood sugar at 1 hour (*milligrams per cent*)	82	92	95	101	121
Calculated CHO + 0.58 P (*per cent*)	69	56	48	38	14

averaged 0.92 to 0.94, a response almost as great as that obtained after a preliminary diet of carbohydrate. Tolerance tests in these animals showed a hyperglycemia which was slightly greater and prolonged for about 2 hours beyond the normal. The respiratory reaction is similar to that found in the long-fasted dog living on its own body protein (p. 266). The respiratory metabolism data indicate that protein in abundance is capable of maintaining the carbohydrate-oxidizing ability of the body, although in a state perhaps somewhat less than optimum. The blood and urine studies show a small diminution in the rate and amount of glucose utilized.

Confirmatory evidence of the glucogenic value of protein in the normal dog is found in some recent data from the tolerance studies by Greisheimer and Hoffbauer (1935). Six animals were tested with 3 grams of glucose per kilogram of body weight after 7 days on diets containing definite amounts of protein, carbohydrate, and fat. In table 1 the relative positions of the tolerance curves are indicated by

the blood sugar levels at one hour after the test meal. The last row in the table gives the calculated total glucose available from carbohydrate plus protein in the diet. This bears a direct relation to the glucose tolerance, whereas the carbohydrate content of the diet without the protein does not. The data discussed in this section lead to the conclusion that the carbohydrate derived from protein should be included with the preformed carbohydrate in attempting a quantitative appraisal of the dietary factors.

Low carbohydrate-high fat diet. Much of the experimental work on low carbohydrate diets has been based on glucose tolerance tests in

TABLE 2

Glucose tolerance tests of normal human adults on low carbohydrate diets

AUTHOR	DURATION OF DIET	DIET			CALCULATED AVAILABLE CHO	GLUCOSE TEST MEAL	BLOOD SUGAR PEAK
		CHO	P	F			
	days	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm. per cent*
Frank and Leiser (1929)	7	19	40		42	400	270
Frank and Leiser	16	19	40		42	350	370
Greisheimer, Goldworthy, and Thomas (1933)	7	29	37	147	65	50	202
Malmros (1928)	1	36	19	184	65	71	180
Malmros	4	39	19	166	67	65	240
Odin (1927)	2	10	80	200	76	200+	270
Malmros (1928)	23	43	19	225	77	70	220
Tolstoi (1929)	365	12	120	250	107	100	250
Tolstoi	365	12	120	250	107	100	210
Himsworth (1935)	7	50	80	240	120	50	202
Himsworth	7	50	80	240	120	50	212
Himsworth (1933)	4	55	90	160	123	50	217
Himsworth	11	55	88	202	126	50	230

human adults. In many cases the diets for the preëxperimental periods have not been expressed in quantitative terms. A number of those in which the dietary carbohydrate, protein, and fat have been reported are assembled in table 2.

All of the experiments show an intolerance when compared with values from control diets under similar circumstances, but it is obvious that the data are not suitable for a quantitative expression on the basis of either the preformed carbohydrate in the diet or the calculated total available glucose, C + 0.58 P + 0.1 F (p. 255).

The predominant position of carbohydrate over fat in determining

the shape of the glucose tolerance curve has been demonstrated recently by Himsworth (1935). He did not consider protein as a factor in these experiments, since the amount (80 grams) was constant in all of the diets. Normal human subjects were tested with 50 grams of glucose and the blood samples taken at 10-minute intervals for 3 hours. Blood sugar curves were compared on the basis of the area above the base line expressed in milligram-minutes. With 50 grams of carbohydrate and 240 grams of fat in the diet the area of the tolerance curve was about 7000 mgm.-min. in a typical subject. There was a marked improvement (area = 3785) when the carbohydrate was increased to 125 grams and the fat decreased to 207 grams. Successive increments of 75 grams of carbohydrate and isocaloric decrements of 33 grams of fat showed improvement at a decreased rate until, with 350 grams of carbohydrate, the area was 2767 mgm.-min. More rapid improvement was again encountered with 425 and 550 grams of carbohydrate. Thus the relationship between tolerance and carbohydrate in the diet could be expressed by an S-shaped curve. Increasing the fat in a constant carbohydrate diet had no effect on the tolerance. Changing the amounts of fat and carbohydrate, but maintaining a constant ratio, likewise indicated that the total fat content of the diet was not important.

Himsworth (1934) has also shown the dietary effect in rabbits by another procedure, a multiple tolerance test in which 1 gram of glucose was given intravenously every 30 minutes for several hours. The animals taking the carbohydrate diet exhibited the same or an improved tolerance with each successive dose, whereas those receiving the fat diet had increasingly higher blood sugars after each injection until at 2 hours the level was about 340 mgm. per cent.

Compared to the blood sugar studies, but little attention has been given to the effect of diet on the ability to oxidize glucose, as determined by the respiratory metabolism. In 1918 Bernstein and Falta noted some delay in carbohydrate oxidation when glucose was injected into subjects receiving either low carbohydrate or high protein diets. Little or no rise in the respiratory quotient was encountered after the oral administration of 100 grams of levulose. The subject was neglected until 1932 when Spencer and McClellan discovered that normal or obese humans partaking of a daily diet restricted in carbohydrate to 25 grams were able to oxidize only about one-half the normal amount of ingested glucose.

An extremely high fat diet of heavy cream, containing about 95

per cent of the calories in fat, was used by Murlin and co-workers in their observations on the specific dynamic action and ketogenic effect of fat and sugar (Murlin, Burton, and Barrows, 1936; Murlin, Nasset, Murlin, and Manly, 1936). After 4 or 5 days of the cream diet the ingestion of 50 or 100 grams of glucose or sucrose produced very little change in respiratory quotient and a definite elevation of the blood sugar at 3 hours after administration.

The effect of an exclusively fat diet for 3 or 4 weeks (125 grams daily of lard or butter) has been studied in three dogs (Chambers and Chandler, unpublished data). Basal respiratory quotients averaged 0.73, and average quotients for 4 hours after the administration of 50 grams of glucose were 0.75. The difference between these results and those obtained by a complete fast for the same length of time (p. 261) is hardly significant. Likewise the excretion of about one-fifth of the test meal of sugar indicates that the glycerol of the ingested fat is no more effective than that from endogenous fat in protecting against the development of hunger diabetes.

A most logical quantitative method of approach has been proposed recently by Johnston (1933). He compared the glucose oxidizing capacity with the "basal available glucose" instead of with the amount of carbohydrate in the diet. "Basal available glucose" was calculated from the amounts of the three foodstuffs oxidized, according to the basal metabolism determination, using the conventional Woodyatt ketolytic formula (carbohydrate + 0.58 protein + 0.1 fat). The glucose oxidizing capacity of five children was obtained from the respiratory data for 4 hours after the intravenous injection of 20 grams of glucose. Different diets were given so that the basal available glucose was varied between 1.26 and 10.44 grams per hour and the amount of carbohydrate oxidized in 4 hours changed correspondingly from 4.88 to 36.43 grams. While the correlation approaches a straight line, the author notes the possible errors involved in calculating hourly metabolism from 8- or 10-minute periods of air collection. The results warrant a more extensive study of this method of calculation, for the few figures given show that the proportions of foodstuffs oxidized in the basal period did not correspond necessarily to the amounts in the diet.

A means of verifying the values used in the Woodyatt formula is suggested by Johnston's work. More complete respiratory metabolism data should place the carbohydrate factor on a quantitative basis, and a wider range of variation in the protein and fat contents of the diet might serve to establish the correct factors for these two latter fractions.

The present protein factor of 0.58 is derived from the D:N ratio of the phlorhizinized dog and of the diabetic human (Lusk, 1928). Data are not now available for validating this factor or substituting 0.44, which is based on the glucose from protein in the depancreatized animal and which agrees with the values calculated from the glucogenic amino acids (Rapport, 1930). The glycerol fraction from fat should not be counted with the carbohydrate, according to the negative results of Himsworth (1935), when the fat content of the carbohydrate constant diet was varied, but the possible effect of the large changes in caloric value of the different diets in his experiments was not considered.

All of the experimental work cited in this section on blood sugar changes, glucose excretion, and respiratory metabolism agrees in showing that a low carbohydrate-high fat diet leads to a diminished ability to utilize sugar.

Low calorie diet. The state of undernutrition brought about by the continued use of a diet inadequate in caloric content apparently has little effect on the utilization of carbohydrate. The earlier literature has been reviewed by Benedict, Miles, Roth, and Smith (1919), by Lusk (1921), and by Morgulis (1923). In the extensive study by the former authors the basal respiratory quotients of a group of young men throughout a period on a low-calorie diet were only slightly lower (average 0.79) than the normal basal level (average 0.80). The loss in body weight was not severe, averaging about 10 per cent. In a more drastic test reported by Morgulis (1923, p. 264) a 40 per cent weight reduction in a dog was brought about by feeding one-third the normal ration for a period of 9 weeks. The basal quotients for the period of restricted diet were about 0.75 except for the last week when a rise to 0.84 was recorded. Thus the body called mainly upon endogenous fat to supplement the caloric deficiency of the food until the fat stores became depleted. However, the carbohydrate in the diet apparently was sufficient to keep the sugar-oxidizing mechanism in a comparatively normal state.

The reaction to ingested glucose has been considered in several clinical studies of undernourished children. Although the basal blood sugar level of marasmic infants may be 10 per cent or more below the normal, their tolerance for glucose is not usually impaired (Tisdall, Drake, and Brown, 1925; M. J. Brown, 1925; Jaso, 1932). The oxidation of administered glucose is normal, according to the respiratory quotients observed by Wilson, Levine, and Gottschall (1928), although their patients exhibited some hyperglycemia and a slight glycosuria

after the test meal. During acute undernutrition young rabbits show a normal tolerance for glucose, according to the blood sugar determinations reported by Sakauchi (1932). The diet consisted of 100 grams of fresh vegetables per day in addition to the daily test meal of 1 gram per kgm. body weight of glucose or levulose or 0.5 gram of galactose. Death occurred in from 6 to 12 days and, in contrast to the fasted animals, was preceded by a marked fall in blood sugar to as low as 21 mgm. per cent in some cases. At this time the liver glycogen was between 30 and 230 mgm. per cent, which is within the range given for the fasting rabbit. The carbohydrate test meals showed a normal blood sugar curve for the three sugars, but an occasional glycosuria was found qualitatively which was attributed to a lowered kidney threshold. In a second series of rabbits a state of chronic undernutrition, lasting in one instance as long as 124 days, was maintained by feeding one-fifth the normal ration in addition to 100 grams of fresh vegetables. Tolerance tests at intervals revealed a slight diminution in utilization of the sugars in the 3rd to 5th week and a marked hyperglycemic reaction with attendant glycosuria in the 6th or 7th week. It is evident that further research is needed to clarify the details in this part of the field. From the present data it seems doubtful that undernutrition *per se* is responsible for any marked interference with the mechanisms for handling carbohydrate in the body.

COMPLETE INANITION. From the standpoint of the changes in metabolism which occur during complete inanition Morgulis (1923) has divided the fasting period into four phases, each corresponding to a loss of about one-eighth of the normal body weight. The first phase represents the transition from the well-fed to the fasting state and is marked by the rapid diminution of carbohydrate stores. During the second and third phases, which are not distinctly separable, the body is deriving energy mainly from the oxidation of fat (80 to 90 per cent) and to a much smaller extent from protein (10 to 20 per cent). When the reserve fat becomes depleted the fourth phase is entered, which is generally characterized by the so-called "premortal" rise in nitrogen excretion, denoting an excessive destruction of endogenous protein sometimes up to 100 per cent of the total energy requirement of the body. With these changes in basic body fuel as the fast progresses one might expect an alteration in the metabolism of carbohydrate. Although the decrease in body weight seems to be a more accurate index of the fasting state than its duration, most investigators have reported their experiments on the time basis. Therefore the data of this section will be considered

according to the length of fast, short, intermediate, or prolonged corresponding in general to the first, second or third, and fourth phases. To facilitate species comparisons it may be considered that the first phase, embodying a weight loss of 12.5 per cent, extends approximately for 2 days in the rat (Dittmar, 1933) and guinea pig (Morgulis, 1923), 4 days in the rabbit, 12 days in the dog (Morgulis, 1923; Dann and Chambers, 1930, 1933), and 15 days in man (Morgulis, 1923).

Short fast. The first phase of fasting, particularly with regard to the assimilation rate as determined by the glucose tolerance test, has been much more thoroughly investigated than the later stages. All species studied have reacted similarly. In the human subject abnormally high blood sugar test curves after a 3-day fast were noted by Traugott in 1922. The same effect was obtained by Goldblatt (1925) and by Goldblatt and Ellis (1932) with a 40-hour fast, and by Sweeney (1927) with a 2-day period. In the 40- to 48-hour fasting experiments the hyperglycemia seldom exceeded 200 mgm. per cent and the glycosuria was only qualitative in extent (Goldblatt, 1925; Malmros, 1928). With a longer fasting period—82 hours—blood sugar values up to 300 mgm. per cent were observed by Heinbecker (1928) in three Eskimos. Likewise in an individual unable to eat for 7 days a peak of 330 mgm. per cent was reached after 50 grams of glucose were administered and 16 grams of sugar escaped in the urine (Malmros, 1928).

In the animal experiments rabbits have been used frequently. In 1925 du Vigneaud and Karr effectively demonstrated that a higher and more prolonged hyperglycemia developed when the test was made after 4 days of inanition. The blood sugar concentration averaged 238 mgm. per cent at the peak and 195 mgm. per cent at 3½ hours after feeding 3 grams of glucose per kgm. to 4-day fasted animals, whereas corresponding figures for a 1-day fast were 190 and 123 mgm. per cent. The fact has been adequately established in this species by Tiitso (1926), by Harding and van Nostrand (1929), and by MacKay, Bergman, and Barnes (1935), and confirmed by the data of Althausen and Thoenes (1932) from a modified tolerance test.

The experimental technique is important. The condition of the animal before the fasting period is started may introduce an error. Plump, well-fed rabbits exhibited a better tolerance after 4 days without food than those which were thin and emaciated (Harding and van Nostrand, 1929). A more detailed study of the preceding dietary effect on 24- and 48-hour fasting periods has been reported by MacKay and Bergman (1933). Although Staub (1922a) noted two cases in which

a more rapid rise in blood sugar was encountered when the glucose was given by duodenal tube instead of by mouth, other investigators have found that the effect is essentially the same whether the sugar is administered orally, intravenously (du Vigneaud and Karr, 1925), or subcutaneously (Tiitso, 1926).

The same general reaction to fasting has been demonstrated by tolerance tests in the dog. Within the longer first phase—about 12 days in this species—a relationship between length of fast and rate of assimilation might be anticipated, but the available data are insufficient for a quantitative expression. The experiments of Kisch, Simons, and Weyl (1929) are invalid for this purpose because the 3-, 7-, and 10-day tests were made during the same fast period and the effect of the previous dose of sugar may last for several days. Deuel (1930) found a tolerance to 16 grams of glucose after 3, 4, and 6 days of inanition represented by a glycemic peak of 194 to 225 mgm. per cent, as compared to about 150 mgm. per cent in the well-nourished controls. There was no consistent distinction as to the length of fast. Holtz (1931) used larger glucose meals than any of the other investigators (213 grams for a 24-kgm. dog) and noted a distinct effect on the 2nd day. The area of the blood sugar curves for the 2-day fasted dogs averaged 770 mgm.-hours, as compared to 370 for those previously fed a carbohydrate meal. Prolonging the fast to 10 and 11 days in two animals did not markedly decrease the tolerance. However, Wierzuchowski and co-workers, with the constant intravenous infusion method, demonstrated a definite impairment in the rate of glucose assimilation after 10 days of fasting, as compared to the postabsorptive state. Dogs in the latter condition, when 2 grams per kgm. per hour were injected for periods of 6 to 30 hours, attained a maximum hyperglycemia of about 250 mgm. per cent in the 1st or 2nd hour. The blood sugar was then maintained at a fairly constant level around 200 mgm. per cent for the balance of the experiment. The urinary loss varied in the different animals between 1.2 and 19 per cent of that injected (Wierzuchowski and Godomska, 1927). After 10 days without food the blood sugar rose with the same test to approximately 500 mgm. per cent in 90 minutes and remained there for 10 hours, during which time about 45 per cent of the infused sugar was excreted. In one animal the impaired metabolism continued for 30 hours of injection, whereas another returned almost to the normal rate by the 23rd hour (Wierzuchowski, 1931b). Some evidence for a graded response is found in the data of Chambers and Marquis (1932). Following the 50-gram test meal the average increase

in blood sugar was 20 mgm. per cent at 18 hours after food; 108 mgm. per cent on the 3rd to the 5th day; and 147 mgm. per cent on the 7th to the 9th day. The glycosuria in the latter group varied between 0.3 and 4.3 grams and averaged 2 grams.

Thus, in the early or first phase of inanition the diminished rate of assimilation of exogenous glucose has been conclusively demonstrated in humans and experimental animals by the data from the blood sugar and urinary excretion studies quoted above. While less information is available concerning the oxidation of the ingested glucose, the few reported determinations of respiratory metabolism indicate a definite inhibition of this function. Goldblatt (1925) found maximum respiratory quotients of 0.81, 0.82, and 0.86 in three human subjects taking 50 grams of glucose after 40 hours without food, and more recently (1932) has confirmed these figures with the added control of a 16-hour fasting experiment in which the non-protein respiratory quotient reached 1.04 in 2 hours. The most comprehensive experiments have been conducted by Cori and Cori (1928) in a comparison of the oxidation and glycogen formation in groups of rats fasted for 24 and 48 hours. The average fasting quotients were the same, 0.715 and 0.713, but for 4 hours after the glucose the non-protein respiratory quotients of the former group averaged 0.90, whereas the figure for the latter was 0.84; thus relatively less sugar was oxidized. The blood sugar concentrations were 158 and 176 mgm. per cent, respectively. Although the longer fast reduced the rate of absorption from the intestines, 0.75 gram per 100 grams of body weight in 4 hours as opposed to 1.065 grams, relatively more of that absorbed was deposited as glycogen. Confirmatory evidence of reduced oxidation has been obtained in the dog (Chambers and Chandler, unpublished data). After 6 days of fast the average non-protein respiratory quotients in three animals following the administration of glucose were 0.86, 0.76, and 0.71, respectively. The low quotient in the latter case, accompanied by a glycosuria of 7.4 grams, is typical of a longer fast. With a longer fast—12 days—in another dog the test quotient was 0.81, and 4.9 grams of sugar were excreted by the kidney. Aszódi and Pélyi (1936) noted similar results with a sucrose test meal. The quotients were 0.82 and 0.81 after a 7-day fast, whereas comparable postabsorptive figures were 1.02 and 1.08.

A contrary opinion has been expressed by Hines, Boyd, and Leese (1929) based on the intravenous injection of 4 grams per kgm. per hour for 2 hours into dogs fasted for 3 to 7 days. The tolerance was less

than that of the controls, according to blood and urinary data, but the respiratory quotients averaged 0.99 as against 0.98 for the fed animals. It seems doubtful if these high quotients are indicative of the carbohydrate oxidized, since there was an average decrease of 12 volumes per cent in the plasma CO_2 content during the course of the experiments. A marked increase in blood lactate is found when glucose is infused intravenously (Wierzuchowski and Laniewski, 1931), but not when given by mouth (Bachmann and Haldi, 1937).

Intermediate period of fast. As the fast progresses into the second and third phases the tolerance test reveals an increasing lack of glucose assimilation. The 18-, 19-, and 22-day periods in rabbits were shown by du Vigneaud and Karr (1925) to produce much higher test hyperglycemias than the 4-day fasts, and the 7- and 14-day experiments appeared in the intermediate positions. Similar results were reported by Eisner (1926) after 9 to 17 days. The intraduodenal administration of glucose to dogs without food for 13 to 21 days was followed by blood sugar values up to 500 mgm. per cent, which lasted for at least 5½ hours (Rathery and Laurent, 1931). Confirmatory evidence is also obtained from data in table 4 (p. 276). Following a fast of 3 to 4 weeks the blood sugar concentration averaged 314 mgm. per cent at 4 hours after the administration of glucose and the average glycosuria was 6.3 grams. These figures may be compared with those from the 7 to 9 day group in the short fasting experiments (p. 260), in which the total blood sugar averaged 217 mgm. per cent and the excretion was about 2 grams.

That the most marked degree of hunger diabetes occurs in the second and third phases of inanition is more clearly demonstrated by the respiratory metabolism than by the tolerance tests. In the classical observations of a human subject by F. G. Benedict (1915) the basal respiratory quotient reached a stationary level by the 15th day. The calorimeter experiments of Anderson and Lusk (1917) demonstrated that by the 13th day of fast in the dog exercise would no longer raise the quotient. During the 4th week of inanition the inhibition of carbohydrate oxidation was almost complete for 4 hours following glucose ingestion (Dann and Chambers, 1930, 1933). After giving 2.5 to 6 grams per kgm. of body weight to four dogs the non-protein respiratory quotients averaged 0.72 for the 2nd, 3rd, and 4th hours. This is in contrast to quotients of 0.80 to 0.85 for the short fasting experiments noted in the preceding section and to the average quotients of 1.00 for the well-nourished controls, in accord with the work of Lusk. It was calculated that up to 1 gram of glucose per hour was oxidized between the 4th and 8th hours

instead of the usual 3 to 5 grams per hour. There is no evidence that the respiratory quotients were not true combustion quotients, since no significant change in the lactic acid concentration or CO_2 combining power of the blood was found. The maximum reduction in urinary ketone bodies in several experiments was equivalent to approximately 10 mgm. of β-hydroxybutyric acid per hour. A retention of CO_2 equivalent to this amount of acid is too small to be determined by the respiratory apparatus.

Protein metabolism. Before discussing the fourth phase of inanition it might be well to examine the data on protein metabolism. In the diabetic organism the impaired metabolism of carbohydrate extends also to the moiety of glucose derived from the glucogenic amino acids, but this fraction has received scant attention in the fasting individual. In 1920 McCann observed the effect of a meal of 350 grams of lean meat and 10 grams of butter on the respiratory metabolism of a patient who had undergone an 8-day fast. The respiratory quotients for the 2nd, 3rd, and 4th hours after the food were 0.69, 0.68, and 0.74, compared to basal quotients of about 0.73. He suggested that the low quotients indicated a retention as glycogen of glucose derived from protein. Since the nitrogen excretion was not increased by the protein-fat meal, a large part of the ingested protein may have been retained as protein rather than immediately metabolized. A meal of 300 to 500 grams of lean meat produced a marked rise in protein metabolism, associated with a slight fall (0.01 to 0.04) in respiratory quotient in two men living on an exclusive fat-meat diet (McClellan, Spencer, and Falk, 1931). The basal respiratory quotients of 0.76 to 0.78 do not indicate as pronounced an inhibition of carbohydrate metabolism as the glucose tolerance tests (Tolstoi, table 2, p. 253).

The glucogenic amino acids, glycine and alanine, were intravenously injected into dogs fasted for 6 to 14 days by Wilhelmj and Mann (1930a). In fifteen experiments on three animals no significant rise in respiratory quotient above the average basal level of 0.69 was found for 4 hours after administration. The average rise in the control experiments on standard diet was 0.05. Due to the slow rate of assimilation the extra nitrogen excretion in most of the experiments amounted to less than 100 mgm. per hour, the equivalent of which, in glycine, would theoretically raise the respiratory quotient by only 0.02 if the glycine were completely oxidized. Considering the variation in quotients between individual experiments, the data are not adequate to demonstrate a lack of oxidation of glucose derived from glycine or alanine. Another

series of experiments with high carbohydrate diets showed that the observed quotients after injection of glycine or alanine were related to the basal respiratory quotients—an index of the state of nutrition of the animal—rather than to the theoretical quotients of the amino acids (Wilhelmj and Mann, 1930b).

Experiments in which large meals of protein have been given to fasted dogs indicate that the glucose formed from ingested protein is retained by animals in this condition rather than oxidized in the normal manner or excreted as in the diabetic organism. After a short fast of 48 hours, when the basal quotient was 0.75, Ralli, Canzanelli, and Rapport (1931) noted a practically normal reaction to 400 grams of meat, i.e., a quotient of 0.79. After 2 more days without food the same protein quotient was found, although the basal level had fallen to 0.73. Evidently the changes in a short fasting period are too small to be demonstrated. The same article presents calculations of the theoretical respiratory quotients in pancreatic diabetes of three proteins—gelatin, casein, and meat—on the basis of their amino acid content. The values are respectively 0.65, 0.78, and 0.73. Upon feeding 100 gram amounts to depancreatized dogs with an average basal level of 0.71, they obtained quotients of 0.69, 0.70, 0.72, respectively. Similar quotients have been found in this laboratory when the three proteins were given to non-diabetic dogs which had fasted for 3 or 4 weeks. Since the data have appeared only in preliminary reports (Chambers and Dann, 1931; Chambers, Chandler, and Barker, 1936), a summary of the average figures is given in table 3. The respiratory quotients are averages for the 2nd, 3rd, and 4th hours after the protein was eaten. Blood samples for sugar were taken at the end of the respiration experiment and the urine then collected for a 5th hour to determine the peak of the nitrogen and acetone elimination. Although a part of the ingested protein was retained as such, over 50 per cent was deaminized according to the nitrogen in the urine, which in some cases amounted to over 500 mgm. per hour at the end of the calorimeter experiment. One indication of the lack of glucose oxidation was the rise of 25 to 50 mgm. per cent in blood sugar, whereas the fed controls showed no significant fluctuations after a meat meal. The respiratory quotients of 0.71 and 0.72 before and after gelatin and lean beef heart are quite similar to those of the depancreatized animals noted above. The apparent rise of 0.04 in the casein quotients may be due to the unusually low basal values. Another diabetic-like reaction is seen in the definite increase in ketone body excretion after meat ingestion.

A similar difference in the reaction of blood sugar to ingested protein has been demonstrated in diabetic patients by Conn and Newburgh (1936). The maximum increase after a meal of lean meat varied from 26 to 75 mgm. per cent in the patients who had a tolerance of less than 100 grams of glucose, while in the normal subjects it was less than 16 mgm. per cent.

It should be noted that the data discussed in this section pertain to exogenous and not endogenous protein. The amount of glucose which may be derived from the low fasting protein metabolism in the intermediate stages of inanition is small (150 to 250 mgm. per hour, table 3). This is utilized by the body, since it is not excreted. Presumably it is oxidized, but the respiratory metabolism method is inadequate to prove the point, since the change in quotient would be of the order of 0.01, an insignificant difference.

TABLE 3

Metabolism of protein in dogs fasted 19–31 days

NUMBER OF EXPERIMENTS, AVERAGED	PROTEIN FED	"BASAL" METABOLISM				AFTER PROTEIN			
		Blood Sugar	Urinary N	Ketone	R.Q.	Blood Sugar	Urinary N	Ketone	R.Q.
		mgm. per cent	*mgm./hr.*	*mgm./hr.*		*mgm. per cent*	*mgm./hr.*	*mgm./hr.*	
3	77 gm. gelatin	56	47		0.71	74	428		0.71
2	100 gm. casein	38	66	1.6	0.68	93	315	1.8	0.72
4	500 gm. meat	52	95	2.9	0.72	75	543	5.8	0.72

Prolonged fast. As the animal passes from the third to the fourth phase of inanition the predominant position of fat as a source of energy is taken over by the body protein. The condition is commonly designated "premortal," which is a misnomer, since recovery is possible. The early studies of this phase are not extensive (Morgulis, 1923, p. 135) and no experiments concerned with the feeding of carbohydrate are recorded. The work of Reicher in 1909 gave some indication of a recovery of carbohydrate oxidation. He noticed a disappearance of fat globules from the blood stream and a decrease in acetone excretion coincident with the premortal rise in nitrogen excretion. The phenomenon did not occur in the absence of the premortal rise and could be reproduced by the administration of sugar. More direct evidence is found in the observations of Morgulis and Edwards (1924) on the progressive changes in the sugar and nitrogenous constituents of the blood

during prolonged inanition. During the first and second phases, when from 20 to 30 per cent of the body weight was lost, the blood sugar was definitely lower than the initial level by some 20 to 30 mgm. per cent. As the weight loss of the dogs reached 40 to 50 per cent there was a rise in blood non-protein nitrogen, creatine, and sugar, with the concentration of the latter surpassing the initial value in some cases. A premortal elevation of the basal respiratory quotient toward the end of a long fast was observed in the wild rat by Benedict and Petřík in 1930. The rise in quotient was correlated with the rise in protein metabolism by Dann (1932) in a 3-day series of experiments on a dog which lost about 50 per cent of its body weight in 36 days. On the 32nd, 33rd, and 35th days the quotients were 0.79, 0.84, and 0.79, respectively, and from the nitrogen excretion it was calculated that 37, 100, and 80 per cent, respectively, of the total metabolism was derived from body protein. This relationship has been definitely established in both the albino and the wild rat by the recent work of Benedict and Fox (1934). In the majority of cases the respiratory quotient rose to approximately 0.80 in the last day or two, but the correlation with the height of the nitrogen excretion was not uniform. In four instances protein accounted for only 22, 29, 31, and 33 per cent of the total metabolism, and the corresponding quotients were 0.80, 0.82, 0.79, and 0.80, whereas in three other cases the protein metabolism was 85, 85, and 88 per cent, with similar quotients of 0.80, 0.81, and 0.80. The majority of the wild rats survived for only 5 or 6 days, but the albinos lived for 7 to 12 days and one for 24 days. The weight loss at death, which varied between 29 and 43 per cent with the one exception of 57 per cent, was less than that reported for dogs.

A closer correlation between the respiratory quotient and the level of protein metabolism was found in the ten dogs recently studied in this laboratory (Chambers, Chandler, and Barker, 1936; Barker, Chandler, and Chambers, 1937). The weight loss was about 50 per cent (45 to 53 per cent), although the duration of the fast varied between 38 and 77 days. In six experiments the nitrogen excretion increased about threefold, and from 60 to 100 per cent of the total basal heat production was attributed to endogenous protein. The basal respiratory quotients varied between 0.80 and 0.84, ketones decreased markedly or disappeared entirely, and the creatine excretion rose to as much as 20 mgm. per hour. In the other four experiments there was little or no premortal rise in nitrogen excretion, the protein metabolism was only 14 to 22 per cent of the total, the ketone excretion remained at approxi-

mately the level of the third phase, and the basal quotients were correspondingly low, i.e., 0.71 to 0.74. Creatine in the urine was elevated to about 10 mgm. per hour. Despite this definite difference in protein metabolism between the two groups, the rise in basal blood sugar was essentially the same, from a level of about 60 mgm. per cent in the third phase to an average of 90 mgm. per cent in the fourth phase. Likewise there was no difference in their reaction to the administration of 50 grams of glucose. The average quotients of 0.86 to 0.92 for the 2nd, 3rd, and 4th hours after glucose ingestion indicate a partial restoration of the glucose-oxidizing function. The recovery was not complete, since a hyperglycemia occurred accompanied by a glycosuria of 2 grams or less in some instances. Glucose derived from ingested protein also was handled in the normal manner, according to the quotients of 0.78 and 0.81 after 500 grams of meat, but ketone bodies again appeared in the urine.

This utilization of carbohydrate is but slightly less than that in dogs which are fed on an exclusively meat diet (p. 252). The similarity in response to glucose suggests that, when the major part of the energy requirement of the body is supplied by protein, whether endogenous or exogenous, sufficient carbohydrate is made available from the protein to provide stimulation for the mechanism of carbohydrate metabolism. The possible glucose derived from protein was between 700 and 1400 mgm. per hour in the premortal stage, as compared to 150 to 250 mgm. per hour in the intermediate stage of fasting (p. 264). The foregoing explanation, based on a high protein metabolism, may apply to the first group which exhibited the premortal rise in nitrogen excretion but would not be valid for the second group in which the body protein metabolism was not elevated nor the acetonuria abolished. Apparently an increased breakdown of tissue protein is not essential for the recovery of glucose metabolism nor for the rise in creatinuria.

The same changes in metabolism have been found in the depancreatized dog. A premortal rise in basal respiratory quotient to as high as 0.84, concurrent with an elevated protein metabolism, was first noted by Hédon (1926). These observations were confirmed and extended by Loubatières (1934). Also in depancreatized cats the basal quotients remained above 0.75 when the loss in body weight exceeded 30 per cent (Ring, 1934), and the diabetic condition could be restored by feeding fat. The change from the third to the premortal stage appeared earlier, and the transition was more abrupt in the depancreatized than in the normal animals, although the same weight loss of about 50 per cent

occurred (Barker, Chambers, and Dann, 1937). For example, in one dog there was no evidence of glucose oxidation on the 19th day of fast, but 3 days later the creatine excretion had doubled without a rise in total urinary nitrogen. The ingestion of glucose then provoked a rise in respiratory quotient, a fall in ketone body excretion, and a recovery of only 40 per cent of the sugar in the urine. Thus the occurrence of the typical premortal reactions in the absence of the pancreas would indicate that the gland is not essentially involved in the processes of restoration of carbohydrate metabolism which take place at this time.

The significance of an increased creatinuria after prolonged fasting is not apparent. It was formerly associated with the destruction of body protein at this time (Howe and Hawk, 1911), but Morgulis (1929) has cited evidence to show that such a relationship is not obligatory. The experiments described above substantiate the latter viewpoint. In some unpublished experiments Barker has found that the creatine excretion of the fasted depancreatized dog may be elevated 100 to 200 per cent by the administration of thyroid gland without correcting the lack of oxidative response to ingested glucose. Ablation of the thyroid, which diminishes the protein metabolism of the diabetic, likewise prevents the premortal rise in total nitrogen, according to Mansfeld and Hamburger (1913) and to the later experiments of Lánczos (1926). On the contrary, Hári (1919) and Mangold (1926) have found that the typical increase may be present in thyroidectomized animals or may be absent in those with the thyroid gland intact. Our results support the latter contention. The evidence seems insufficient at present to assign to the thyroid a controlling position in the protein metabolism of the fourth phase of inanition.

GLYCOGENESIS. *Effect of diet.* Glycogen formation is an important part of glucose utilization, since in the normal individual the rate of oxidation may be considerably less than half the rate of absorption of the ingested sugar. Most of the literature concerning glycogenesis has dealt with formation from the different sugars or the intermediary substances within a few hours after their administration. There has been little effort to correlate glycogen data with the reduced glucose assimilation provoked by a low carbohydrate diet. In the Herter Lectures, Macleod (1934) noted that the preceding diet influenced the percentage of glycogen in the liver and possibly in the muscle, but he could offer no definite explanation for the observed variations. After discussing the possible errors in glycogen determinations he concluded that but few of the recorded investigations prior to that time could be regarded

as strictly accurate. The recent work shows that the glycogen content of the liver varies in a general way with the available carbohydrate in the daily ration. Rats kept on a diet containing 82 per cent of sucrose averaged 6.03 per cent of liver glycogen; on a 95 per cent casein diet, 2.02 per cent; and after an 80 per cent lard diet, 1.79 per cent (Greisheimer and Johnson, 1930). Growing animals taking a carbohydrate-free diet of casein and lard did not show variations consistent with the protein content of the diet (Greisheimer, 1931). In a recent summary Bollman and Mann (1936) reported values for dogs 18 hours after food. The figures for the carbohydrate diet are somewhat higher than those given above for rats, and the difference between the protein and fat effect is greater. The minimum and maximum liver glycogen content for the different dietary groups was as follows: carbohydrate diet, 7.12 to 11.05 per cent; standard diet, 4.73 to 6.74; protein, 2.26 to 5.90; fat, 0.07 to 2.32; 12th to 21st day of fast, 0.29 to 2.10 per cent. Muscle glycogen seems to be much less susceptible to dietary changes. Appreciable differences were claimed by Sahyun, Simmonds, and Working (1934) when the carbohydrate in the diet was varied between 54 and 77 per cent, but Blatherwick, Bradshaw, and Sawyer (1936) were unable to confirm the experiments. The rats were kept on the diets for 7 or 8 weeks and fasted for 24 hours before analysis of the extensor and flexor thigh muscles. The slight difference between the dietary groups was not in accordance with the carbohydrate content of the diet.

The controversy over the possible transformation of fatty acid into carbohydrate has stimulated considerable interest in the effect of a pure fat diet on glycogen deposition. A number of investigators have reported an increase in liver glycogen after feeding fat, although the figures are far from consistent (Takao, 1926; Burn and Ling, 1928; Bodo and Marks, 1928; Magnussön, 1929; Greisheimer, 1931; and Dittmar, 1933). Gregg (1931) analyzed the balance of the rat carcass, as well as the liver, for glycogen plus free sugar and demonstrated that the three- to fourfold increase in the liver after 3 to 8 days of butter intake amounted to a rise of only 22 per cent in the total glycogen content of the body. Two per cent of the ingested glycerol could account for the deposited glycogen, and the respiratory metabolism gave no indication of its formation from the fatty acid fraction. Similar results were obtained by Fürth and Majer (1932) through varying the daily ration of lard from 0 to 35 calories per 100 grams of rat. After 5 to 7 days on the high fat diets the total carbohydrate of the liver ranged between 0.8 and 2.4 per cent, corresponding roughly to the fat intake.

An abrupt fall in liver glycogen on the 1st day of the butter diet was noted by Gemmill and Holmes (1935), which was followed by a gradual rise for several days. In the dog the duration of the fat diet between the 5th and 15th days did not affect the liver glycogen values (Bollman and Mann, 1936). The fluctuations between 1.25 and 5.16 per cent were all lower than the 5.75 per cent average for the controls. Bickenbach and Junkersdorf (1928) also noted a reduction when the fat diet was given for several days after an 11-day period of fasting. Studies of liver glycogen formation in rats from single meals of fats have been reported recently by Deuel, Butts, and co-workers. Triglycerides of fatty acids in the even numbered carbon atom series from 2 to 8 yielded an amount of glycogen which could be attributed to the glycerol fraction, but there was none from the natural fats of 14 or more carbon atoms (Deuel, Butts, Blunden, Cutler, and Knott, 1937). The 3-, 5-, and 7-carbon acids were definite precursors of glycogen. Extending the series to oleic and undecylic acids in the ethyl ester form gave the same distinction, namely, glycogen formation from the odd but not from the even carbon acids (Butts, Blunden, Goodwin, and Deuel, 1937). The data on fat diets indicate that the concentration of glycogen in the liver may be increased by the ingestion of fat, but it has not been demonstrated that the even carbon fatty acids participate in the glycogenesis.

The effect of the diet on the ability to form glycogen from sugar has been studied in several ways. Kageura (1924a, b) performed the test by perfusing the liver for 1 hour with glucose in defibrinated blood. Analysis of the tissue showed a deposition of glycogen if the dogs were fed previously with an abundance of carbohydrate but none after 2 days on a fat meat diet. The source of the perfusing blood did not alter the results. With the intact animal MacKay and Bergman (1933) have arrived at the opposite conclusion. Three groups of rats were maintained for 45 days on different diets, one containing 65 per cent protein, another 50 per cent carbohydrate, and the third 44 per cent fat. As in the data quoted above the diet had no significant effect on the content of muscle glycogen either 24 or 48 hours after the last meal, nor was the amount formed in 5 hours after glucose ingestion appreciably altered by the diet or by the length of fast. The liver glycogen of the protein fed animals was low at 24 hours of fast and high at 48 hours, but there was little apparent difference in the rate of glycogenesis. Sucrose has been used for the test meal by Abelin (1935) in a series of experiments on the effect of the fat component of the diet. The fats

were fed separately or added to the regular food for several days and the liver and muscle glycogen of the rats determined 8 hours after sugar ingestion, which was preceded by a 16-hour fast. With the less saturated liquid fats—olive or almond oil—both the liver and muscle glycogen after the test meal were definitely higher than the controls and similar to the values obtained with a fast of 40 hours. Liver glycogen was lower than the controls when sucrose was substituted for fat. Butter showed little effect. It was concluded that the fats rich in saturated fatty acids were less effective, although the lowest amount in the liver was found in the cod liver oil series. Control experiments on the glycogen content of the animals before the sugar was fed would be instructive.

Glycogenesis in fasting. The data on changes in the glycogen content of different species during inanition recently have been collected into a summary table by Ling and Shen (1934). A gradual fall in muscle glycogen as the fast progresses has been a common observation, but some disagreement appears concerning the behavior of the liver, particularly in the earlier stages. Several investigators have reported an increase on the 2nd day of fast in rats. In Dittmar's (1933) series on male rats, with a loss in body weight up to 30 per cent, there was no difference between the amounts at 24 and 48 hours, but definitely higher levels of glycogen concentration were found in the groups fasted for 3 to 7 days. A larger group, studied by Deuel, Gulich, Grunewald, and Cutler (1934) for sex differences, showed a gradual fall to a minimum of 0.31 per cent in the male and 0.13 per cent in the female at 72 hours, followed by some increase in both sexes in the next 2 days. The authors explain the high 24-hour level on the basis of the abundant carbohydrate intake before the fast. The increase occurred between the 3rd and 7th day of fast in the experiments of Wetzel and coworkers (1936). It seems likely that the rise in the intermediate stage of inanition is a reflection of the metabolism of endogenous fat in a manner similar to that of ingested fat noted in the previous section.

There are also conflicting opinions as to the ability of the fasted animal to form glycogen from sugar. When Johansson (1909) found evidence of a diminished oxidation of administered glucose he suggested that the sugar goes exclusively to glycogen if the glycogen stores have been decreased by fasting. The opposite viewpoint was soon proposed by Bang (1913), i.e., that a glycogen depleted liver is unprepared to form glycogen at a normal rate. The latter hypothesis has been supported by several investigators. Barrensheen (1913) determined the glycogen content of the liver after perfusing it with glucose for about

1 hour. In the two rabbits fasted for 6 days the increase in liver glycogen of 1.01 and 0.13 gram is not consistent but is definitely less than the 2 gram rise in the two well-fed controls. An impairment of glycogenesis from glucose and galactose, but not from fructose, was reported by Nagasuye (1925). Control sections of the liver from dogs fasted for 3 days showed glycogen contents of 0.70 and 0.85 per cent. The analyses averaged after glucose about 0.60 per cent, galactose about 0.70, and following fructose up to 2.8 per cent. Since only 20 grams of sugar were given and the tissue samples were not taken until 8 or 10 hours later, it is probable that, with this short fast, a considerable portion of the glucose was oxidized. According to the data of Eisner (1926), in rabbits which were fasted for 9 to 17 days and killed 3 hours after 35 grams of glucose, the liver contained less than 0.65 per cent of glycogen, whereas at 24 hours about 3 per cent was found. Such a delayed formation is difficult to interpret, particularly since no glycogen was found in the heart, muscle, or kidney. An increase in liver glycogen from an average content of 0.34 per cent at ½ and at 1 hour to 1.05 per cent at 2 and at 3 hours, after the administration of 3 grams of glucose per kilogram, would indicate deposition in dogs fasted for 11 days (Junkersdorf and Mischnat, 1930). The evidence is less positive when the figures are compared with 0.90 per cent in the four control animals. The striated, smooth, and cardiac muscles showed no significant changes.

Recent experiments demonstrate that the ability of the fasted animal to synthesize glycogen is not as greatly impaired as the foregoing data would indicate. In a series of 24-hour and 48-hour fasted rats Cori and Cori (1928, 1931) made a complete balance study of the relative absorption, oxidation, and changes in the glycogen content of the liver and of the whole body (p. 260). In the longer fasted animals absorption was diminished about 25 per cent and the absolute amount of glycogen deposited in 4 hours was therefore smaller, but on the basis of the amount of sugar absorbed there was relatively less oxidized and more deposited as body glycogen than in the 24-hour group. The relative figures for liver glycogen were about the same. The experiments of Deuel, MacKay, Jewel, Gulick, and Grunewald (1933) include separate analyses of the leg muscles and liver in rats fasted 48 hours. A significant increase occurred in muscle glycogen from a level of about 0.15 per cent in the controls to about 0.25 per cent after glucose, and 1 per cent or more of glycogen was deposited in the liver. More definite evidence against the theory of impaired synthesis is found in the previously cited data of MacKay and Bergman (1933). The amount of liver glycogen

formed from glucose in 5 hours averaged 2.88, 3.18, and 3.10 per cent, respectively, for the three groups which fasted 48 hours, as compared to averages of 2.07, 2.43, and 2.65 per cent, respectively, for the 24-hour series, but glycogenesis in the striated muscle was not significantly altered in this time. Similar results for the short fasting period have been obtained by Meyer (1934) with a fructose test meal.

The few experiments on the second or intermediate stage of inanition show no diminution in ability to form liver glycogen but a possible decrease in this function in the muscle. Determinations before and after glucose in the same individual dogs, fasted about 4 weeks, were made by Dann and Chambers (1932). Control samples of liver and leg muscle were removed under amytal anesthesia, and several days later the process was repeated 4 or 6 hours after the administration of glucose. The tissues were analyzed for glycogen, soluble carbohydrate, and lactic acid. Absorption of the glucose was practically normal, being 0.8 and 1.0 gram per kilogram per hour in the two cases determined. Liver glycogen increased from an average for the three animals of 0.78 per cent in the control samples to 3.65 per cent after sugar. Changes in total carbohydrate plus lactate were in proportion to the glycogen figures. In the leg muscles the average for the first control samples was 0.39 per cent and for the second ones, several days later, was 0.31 per cent. After glucose the average was 0.40 per cent, an increase of 0.09 per cent over the second control. If the fall between the first and second control is extrapolated to the time that the sugar was administered, the glycogenesis would be greater. These figures are very close to those for the shorter fast period of 6 days given by Deuel and co-workers (1933). The average glycogen content in seven dogs was 0.45 per cent in the liver and 0.31 per cent in the muscle. Six hours after glucose the averages were 3.94 per cent for liver and 0.49 for muscle, and 6 hours later they were 3.34 and 0.51 per cent. The experiments of Meyer (1934) with fructose indicate a greater liver glycogen formation in the 96-hour than in the 48-hour fasted rats, but the increase in muscle glycogen was definitely less in the second stage of inanition.

Available data on the fourth or premortal phase of inanition are not sufficiently consistent to permit any conclusive statement about glycogen content or formation. The problem is an important one, since the rise in blood sugar and the partial restoration of glucose oxidation do not seem to be dependent on an increased protein catabolism. In Pflüger's experiments (1907) the two dogs which fasted 70 and 73 days

with a weight loss of almost 60 per cent had 1.22 and 0.03 per cent of glycogen in the liver and almost undetectable amounts in the muscle. Michailesco (1914) took exception to Pflüger's statement that glycogen persists in the body until death and claimed that it disappeared completely in the majority of his dogs after fasting for 10 to 22 days. The longest survival was 38 days. Low values for glycogen and true sugar concentrations of about 0.06 per cent in 10- and 12-day fasted rabbits were reported by Matsuura (1933), but the animals were killed by bleeding. No glycogen was found by Schönfeld (1935) in three rabbits fasted up to 5 days. Considering the careful technique which has recently been shown by Evans, Tsai, and Young (1931) and by Cori and Cori (1931) to be obligatory in the prevention of glycolysis, it seems doubtful if such low concentrations represent the actual condition in the body. Contrary to Michailesco, the livers of four dogs that lost 40 per cent of the body weight in 40 days averaged 0.64 per cent of glycogen, although only traces were found in the muscle (Nitzescu and Benetato, 1928). Likewise the analysis of two dogs after 43 days revealed an average glycogen content of 0.46 per cent in the liver and 0.11 per cent in the striated muscle (Junkersdorf and Mischnat, 1930). In three of the ten dogs in the premortal condition studied in this laboratory (p. 265) liver and muscle tissue were analyzed 4 hours after the administration of glucose for glycogen and for total fermentable carbohydrate by the acid hydrolysis method (Cori and Cori, 1933). The rate of absorption was normal in two of the animals (0.95 and 1.18 grams per kgm. per hour) but low (0.58) in the third one. There was a wide variation in carbohydrate content among the different dogs, but most of the figures were lower than the comparable values in the intermediate phase of fasting (p. 272). Glycogen in the liver was between 0.32 and 2.55 per cent, less than 0.20 per cent in the leg muscles, and from 0.29 to 1.01 per cent in the heart. The amounts of total carbohydrate were proportionally higher. It seems likely from the available information that the carbohydrate content of the tissues, particularly striated muscle, may become markedly depleted by prolonged fasting. Glycogen formation in the liver may be almost normal but in the muscle its deposition is more questionable.

Carbohydrate compounds. A few exploratory experiments have been conducted in the field of the substances other than glycogen which might be involved in the formation or metabolism of glucose. Determinations of glycogen and hexosemonophosphate were made by Wesselkina (1932) in connection with a study of the other phosphate fractions in the leg

muscles of cats fasted until a loss in body weight of from 20 to 43 per cent occurred. In the well-fed controls the average value of 24 mgm. per cent for hexosephosphate phosphorus is considerably higher than the average of 8.9 mgm. per cent in the rat (Cori and Cori, 1931). The average glycogen content was 688 mgm. per cent. The usual fall in muscle glycogen during a fast was encountered (the average content was 281 mgm. per cent) and was accompanied by a decrease in hexosephosphate phosphorus to 9 mgm. per cent. Similar values were obtained in depancreatized cats. An elevation of the hexosephosphate with the glycogen, when glucose, fat, or insulin is administered to the fasting cat, has been reported by Jakovlev (1935). The phosphate levels were 9 to 10 mgm. per cent in fasting; 19.5, 2 hours after sugar ingestion; 33 after repeated doses of fat, 80 after the injection of insulin. The disagreement between these results and those of Cori and Cori (1931) on the normal rat needs elucidation before the described changes can be attributed to inanition.

Another substance of interest in the fasted animal is the so-called "bound" or "protein sugar," obtained by subtracting the free sugar from the total reducing material of the blood after hydrolysis with dilute acid. The early literature is reviewed by Grevenstuk (1929). The high value in the blood of long-fasted dogs (Bierry, Rathery, and Laurent, 1931) suggests interesting possibilities that call for further study.

FACTORS INVOLVED IN UTILIZATION. To summarize the literature quoted thus far, the utilization of carbohydrate by the tissues appears to be adjusted quite accurately to the bodily supply of sugar, either actual, or potential in the form of protein. The effect of comparatively small decreases in the carbohydrate content of the diet or of a short period of fast is reflected in the hyperglycemia of a diminished glucose tolerance. More drastic dietary changes or longer fasting periods reduce the utilization until a part of the ingested sugar is lost from the body by excretion. In the premortal stage sugar is furnished from endogenous protein and the ability to utilize it is regained. The unexplained exception is the premortal condition in which there is no rise in protein metabolism. Thus the minimum utilization in the fasted animal is found in the intermediary stages of inanition. What processes are involved in the adjustment of the body to the changes in the carbohydrate supply? Can the decreased metabolism of ingested carbohydrate be explained as a depression of oxidation, a reduction in the rate of glycogenesis, or should some other form of sugar utilization be sought?

Oxidation and glycogen formation. With a restricted carbohydrate diet or a short fasting period all of the respiration experiments show a subnormal rate of oxidation, which could account for the decreased tolerance. While the glycogen data are not so definite, there is little reason to believe that a diminished glycogenesis is a factor here but, on the contrary, a greater deposition of glycogen probably occurs to offset partially the fall in oxidation. This is supported by the only studies in which both respiration and tissue determinations were made on the same animals, those of Cori (1931, p. 236), comparing the 48- with the 24-hour fast in the rat. In the longer fasting group the lower non-protein respiratory quotient after sugar, 0.84 instead of 0.90, denoted that 38 instead of 44 per cent of the absorbed sugar was oxidized. The decreased oxidation was balanced by a greater deposition of glycogen, mainly in the tissues other than the liver, so that the total recovery of glucose was the same, about 90 per cent in both groups. Therefore utilization could be considered as practically complete.

In the second stage of inanition the exaggeration of the same processes introduces other factors. The reactions in the fasted animal during 4 hours after the administration of glucose may be contrasted with those in the normal well-fed animal. The latter exhibits but little hyperglycemia and no glycosuria. From the total heat production and the respiratory quotient of 1.00 it can be calculated that about 40 per cent of the absorbed sugar is oxidized and the remaining 60 per cent is presumably transformed into glycogen. In the fasted dog which has the same rate of absorption during this time the blood sugar may reach and maintain a concentration of 300 to 400 mgm. per cent and thus produce a glycosuria of 1 to 2 grams per hour. This represents an absolute reduction in utilization of 10 to 20 per cent from the normal. The respiratory quotient remains at the basal level of about 0.72, thus the 40 per cent normally oxidized must be disposed of in some other manner. The loss in the urine would account for one-half or less of the unoxidized 40 per cent fraction. Glycogen deposition is apparently normal in the liver. In the striated muscle glycogenesis may be subnormal, although additional data are needed to clarify the point. Another method of temporary utilization, dependent on the hyperglycemia, is the retention of glucose as such in the tissue fluids, particularly in the skin (Folin, Trimble, and Newman, 1927). To what extent the accumulation occurs has not been determined. The amount in the skeletal muscle probably is not an important part of this fraction, for

the average muscle sugar was only 86 mgm. per cent when the blood sugars averaged 231 mgm. per cent in the analyses of Dann and Chambers (1932). We therefore see in this stage a diminished utilization of ingested glucose leading to some loss by excretion, brought about by a lack of oxidation and a probable decrease in ability to form muscle glycogen and involving a temporary storage in some of the body fluids.

Corroborative evidence for the reduction in oxidation is found in the reaction of the fasted animal to repeated doses of glucose. Table 4 summarizes the results obtained in this laboratory. The quotients are averages of the 2nd, 3rd, and 4th hours after glucose, the blood sugar figure shows the level at the end of the 4th hour, and the urinary sugar is the amount excreted in the 4-hour period. After the second glucose meal about 2.5 grams of glucose were oxidized per hour and the excretion

TABLE 4

Average metabolism of glucose in five dogs after fasting for 22–31 days

NUMBER OF EXPERIMENTS	GLUCOSE FED	NON-PROTEIN R.Q.	BLOOD SUGAR	SUGAR EXCRETED IN 4 HOURS
			mgm.	*grams*
4	1st 50 gm.	0.72	314	6.3
4	2nd 50 gm.	0.86	262	1.5
5	3rd 50 gm.	0.92	180	+
3	4th 50 gm.	0.94	90	0
2	5th 50 gm.	0.88		0
2	6th 50 gm.	0.92		0

fell to about 0.4 gram per hour. By the fourth dose the respiratory quotients and the glucose tolerance were almost normal and the glycosuria had disappeared. Thus the restoration of the oxidative function, as judged by the respiratory quotient, was directly correlated with the gradual recovery of the tolerance for glucose according to the blood and urinary data. The beneficial effect of repeated doses of glucose is found in the phlorhizinized but not in the depancreatized animal.

A contrary opinion has been expressed recently by Soskin and Mirsky (1935b), who have concluded from their observations on the fall in blood sugar of eviscerated dogs, that there is no suppression of carbohydrate oxidation in fasting.[1] Some of the animals were fasted for 2 days and

[1] Since this was written, Bergman and Drury (Proc. Soc. Exper. Biol. and Med. **37**: 414, 1937) have shown that fasting reduces the rate of sugar utilization in the eviscerated rabbit when tested by the continuous injection method for 4 hours. An average of 110 mgm. of glucose per kgm. per hour was used by the group fasted for 4 to 6 days, as compared to 206 mgm. for the fed controls.

others for 3 or 4 weeks. The blood sugar changes for 2 hours after evisceration under nembutal anesthesia and for 2 additional hours after an intravenous injection of glucose were practically the same in both groups. The ureters were ligated, thus no sugar could be excreted, and all of the injected sugar must be utilized in some manner. It is questionable whether the results of such acute experiments can be applied to the normal intact animal. The sudden hyperglycemia of an intravenous injection is not comparable to the prolonged hyperglycemia maintained during the continuous absorption of glucose from the intestine over a period of several hours. Considering the possibility of retention of sugar in the tissue fluids when the blood sugar is high and a gradual return of the same to the blood as its sugar concentration falls, also the diffusion of lactate in the tissues, it seems doubtful that the blood sugar changes alone can be used as the criterion of oxidation.

Liver. It is not surprising that conditions affecting the liver should be reflected in the glucose tolerance of the body. To what extent the liver rather than the other tissues can be held responsible for the reduced utilization during inanition cannot be decided from the available data. Hepatic damage experimentally produced by phosphorus or chloroform poisoning (Althausen and Thoenes, 1932) or by bacterial toxins (Soskin and Mirsky, 1935a) has led to an increased tolerance in some instances and a reduction in others, varying with the progress of the toxic action and the amount of sugar used for the test. The results are difficult to interpret because a quantitative expression of the degree of hepatic damage or evidence that it is confined to the liver has not been obtained. The importance of the nutritional state of the liver in influencing its detoxicating function has been discussed recently by Bollman and Mann (1936).

Dogs with an Eck fistula which were fasted for 50 hours and given glucose *per os* exhibited a higher or more prolonged hyperglycemia than the unoperated controls (Gebhardt and Fricke, 1930). In the hepatectomized dog a reduced tolerance has been demonstrated for continuously injected glucose by Pollack and co-workers (1933, 1934) and for a single intravenous injection by Soskin, Allweiss, and Cohn (1934). The former authors noted blood sugar concentrations of about 500 mgm. per cent when glucose was injected at the rate of 2 grams per hour per kgm. body weight and a retention or assimilation of 0.6 to 1 gram per kgm. per hour. This reduction of 50 per cent or more in the rate of assimilation lowers the glucose metabolism to the level of or a little below the normal amount to which the animal is subjected by absorption from the intestines. The reduction after hepatectomy is much greater than

the amount taken up by the liver in the 28-hour fasted dog (about 21 per cent of the total injected), according to the calculations of Wierzuchowski and Fiszel (1935) from the concentrations of sugar in the blood flowing through the different organs when the same injection technique was employed. The intolerance of the hepatectomized dog is similar to that obtained with the infusion method by Wierzuchowski (1931b) in dogs fasted for 10 days, which is in the early stage of inanition when the inhibition of carbohydrate metabolism has not reached the maximum. However, the fact that a similar effect was produced by the constant infusion of adrenin with glucose (Wierzuchowski, 1931b) raises a question as to the significance of the comparison. The hepatectomy experiments emphasize an important function of the liver in assimilating a rapid inflow of glucose, but in view of the data on oxidation and glycogen formation they can hardly be interpreted as localizing in the liver the entire inhibitory effect of fasting.

Pancreas and insulin. Soon after the discovery of insulin T. B. Robertson (1923), in commenting on Southwood's observations (1923), credited the pancreas with the controlling influence in hunger diabetes. An unexplained inhibitory effect with insulin was found by du Vigneaud and Karr (1925) in both 1- and 4-day fasted rabbits. The diminished tolerance occurred if the test was made when the blood sugar level returned to normal following an insulin hypoglycemia. The more recent experiments of other investigators indicate that a partial restoration of normal carbohydrate utilization after inanition is effected by giving the insulin with the glucose test meal or 24 hours previously.

In the experiments of Cori and Cori (1926, 1927) the addition of insulin after 2 days of fast raised the post-glucose non-protein quotient to 0.90, equivalent to an oxidation of 0.378 gram of glucose in 4 hours, as compared to 0.281 and 0.131 gram, respectively, in the rats with a mild or severe ketosis and receiving no insulin. The increased oxidation was balanced by a diminished glycogenesis in the liver. The 24-hour fasted rats without insulin likewise exhibited quotients of 0.90 and 0.92 with the hormone. The lack of complete recovery was more clearly defined in the longer fasting experiments on the dog (Dann and Chambers, 1930, 1933). Partial oxidation of the first dose of glucose, when administered with insulin, was shown by the average respiratory quotient of 0.79, although hyperglycemia and glycosuria were present. Restoration of oxidation was practically complete with the second test meal, but the glycosuria did not disappear until the third one. Six units of insulin per kilogram were no more effective than 2 units. In the human subject, 39 hours after the last meal, Goldblatt and Ellis

(1932) observed a dissociation of the oxidative and glycemic effects of 10 units of insulin, in that the blood sugar curve after glucose was normal, but the elevation of the respiratory quotient was definitely subnormal. Ellis (1931) also noted that insulin corrected the impaired tolerance produced by a ketogenic diet. Further evidence of incomplete recovery with insulin is derived from Wierzuchowski's constant infusion experiments (1931b). Dogs which had fasted for 10 days excreted up to 45 per cent of the injected glucose during the first 5 hours, whereas with the addition of insulin the excretion was only about 25 per cent. The influence of the hormone was not apparent after the first 5-hour period, since as much as 50 per cent of the sugar was excreted in the next 5 hours irrespective of insulin infusion. After a protein diet the improvement with insulin was less than 5 per cent for the 10-hour period. It is evident from these various experiments that insulin is capable of partially restoring the defective glucose utilization, which fact seems to fit the theory that the endocrine function of the pancreas becomes suppressed by a lack of carbohydrate.

Another mechanism also has been suggested, namely, a change in the sensitivity of the body to insulin, usually inferred from the fall in blood sugar following injection of the hormone. The early experiments indicated that animals fed on a carbohydrate-free diet showed a diminished blood sugar response to insulin (Abderhalden and Wertheimer, 1924; Bainbridge, 1925; Hynd and Rotter, 1931), although Gilchrist (1932) reported an increased reaction in children on a ketogenic diet. The fasting of rabbits for 1 or 2 weeks induced a less abrupt fall in the blood sugar during 1 hour after insulin (Tiitso, 1925). The most extensive studies have been made by Himsworth (1933, 1934, 1935). He noted that the rapid fall in blood sugar after insulin when an individual received a high carbohydrate diet could be reproduced by giving several glucose meals before the insulin test. The same distinction between the effect of a carbohydrate and a fat diet was found when the insulin was given to rabbits during a series of consecutive glucose tolerance tests. In the human subject the injection of the hormone was followed by a latent period before the fall in blood sugar began, and a second dose 13 minutes after the first one also exhibited the same delayed effect. Under the dietary régime used for the glucose tolerance tests (p. 254) the latent period was found to vary from 2 minutes with the 500 gram carbohydrate diet to 7 minutes with the 50 gram carbohydrate diet. Considering only the first 15 minutes after injection, the area of the insulin depression curve likewise was proportional to the carbohydrate in the diet and was directly correlated with the glucose

tolerance curves. Himsworth has proposed the theory that the latent period is an expression of an activation of insulin and he concluded that the dietary variations in glucose tolerance can be attributed to a change in sensitivity to insulin rather than to differences in pancreatic output.

In this connection it should be noted that glucose tolerance and insulin sensitivity are not always so closely associated. After removal of the pituitary gland in the dog approximately the normal fall in blood sugar may be obtained with one-tenth the amount of insulin, although the glucose tolerance may not be significantly altered (Houssay, 1936). A relationship between sensitivity and the amount of available glucose is suggested by studies of diabetes. An increase in the glucose equivalent of a unit of insulin when extra glucose is added to the diet has been noted in the depancreatized dog (Allan, 1924; Cuthbert and Ivy, 1935) and in the diabetic human (Newburgh and Waller, 1932), although the results of Bollman and Mann (1934) are not in agreement. To what extent this may apply to the carbohydrate stores in the fasted animal is unknown. Another interesting aspect of the problem has been approached by Sweeney and associates (1928, 1934) through the action of diphtheria toxin. The daily administration of glucose to otherwise fasted toxemic rabbits resulted in a gradually diminishing tolerance for 6 days, which was not corrected by the addition of insulin. A detailed discussion of insulin sensitivity is contained in the review of Wilder and Wilbur (1937).

The interpretation of insulin sensitivity from the blood sugar changes involves a number of difficulties. Himsworth has emphasized the value of selecting test curves which start at the same level, but this is impossible when the exaggerated condition of a pure fat diet or of the second stage of inanition are compared with the normal well-nourished state. During a fast of 3 weeks, or an equal period on a daily diet of 100 grams of lard only, the basal blood sugar concentration in the dog gradually fell from an average level of 77 to an average of 60 mgm. per cent (Chandler, unpublished data). The decrease varied in different individuals between 10 and 25 mgm. per cent. When the animals were postabsorptive after the standard diet, $\frac{1}{4}$ unit of insulin per kgm. body weight produced a fall in blood sugar of 30 to 40 mgm. per cent in 15 to 30 minutes, with a gradual return to the original level after 90 to 120 minutes. After the fast or fat diet the decrease following insulin was less abrupt, amounting to between 20 and 30 mgm. per cent in the same time and reaching a minimum at about the same level, or in some

cases a few milligrams per cent below the normal. Between 30 and 120 minutes the curves were practically the same, irrespective of diet or fast. The essential difference is in the initial level and in the reaction during the first 30 minutes. From a careful analysis of a large volume of data on different doses of insulin in rabbits Scott and Dotti (1932) conclude that the percentage fall is a more accurate expression than the absolute fall or the minimum level. On this basis fasting or fat feeding for 3 weeks diminishes the initial reaction to insulin.

The fall in blood sugar within 30 minutes is a very rapid manifestation of the action of insulin compared to the duration for several hours of the change in the respiratory metabolism or the probable period of glycogen transformation in the tissues. It is possible to demonstrate the effect on the oxidation of a glucose test meal for several days after the insulin injection. In the experiments just described the fasted dogs were given the 50 gram glucose test at intervals of 1 to 6 days following the insulin experiment. No distinction in respect to the rise in respiratory quotient could be made between administering the glucose simultaneously with the insulin and 1, 2, or in some cases 4 days later. Since in these experiments the insulin has exerted its initial hypoglycemic effect when injected, it is possible that the delayed action on oxidation represents a secondary reaction, such as the transformation of glucose into an oxidizable form, perhaps glycogen, the oxidation of which is stimulated when glucose is later administered. The retention of some of the insulin in an inactive state seems to be a less plausible explanation because in the depancreatized dog, after the 3rd day following the removal of the gland or cessation of insulin injections, there is no rise in respiratory quotient when glucose is administered (Barker, Chambers, and Dann, 1937).

These data lead to the conclusion that insulin is one of the factors in the mechanism of the deficient glucose utilization in hunger diabetes. Its value in effecting recovery becomes diminished as the severity of the condition increases. The fact that large amounts are no more efficacious than small ones favors the theory of a change in the sensitivity of the body as the fast progresses rather than a reduction in the secretory function of the pancreas. But with the present methods an accurate measurement of insulin sensitivity probably is hindered by the compensatory reactions and perhaps involves the amount of carbohydrate substrate available in the tissues.

Hypophysis. The antidiabetic effects of hypophysectomy in pancreatic and phlorhizin diabetes suggest an ameliorating influence in

hunger diabetes. The study of inanition in the absence of the pituitary is complicated by the susceptibility of the animals to hypoglycemic shock. Urine was collected from three adult dogs by Aschner (1912) for a 4-day fast period before and after hypophysectomy. After the operation the glycosuria induced by injection of adrenalin was much reduced and the endogenous nitrogen excretion was decreased to about two-thirds of the normal amount. Confirmatory results were obtained with young litter mates. In a more detailed study of the low protein metabolism Braier (1931) fasted seventeen dogs for various periods up to 13 days. The blood sugar fell rapidly and hypoglycemic crises developed between the 6th and 12th days. Puppies between 5 and 10 weeks old are very susceptible to hypoglycemia hypophysiopriva shortly after the operation, according to the data of Mahoney (1934). The glucose tolerance of two hypophysectomized dogs was determined at 4, 8, and 14 days of inanition by D'Amour and Keller (1933). Absence of the pituitary gland did not improve the tolerance, since the test hyperglycemia was higher and more prolonged than in the normal control animals. Some observations on longer fasting periods, noted in a preliminary report from this laboratory (Chambers, Sweet, and Chandler, 1935) have been extended to include twelve dogs. The pituitary glands were removed intact (Sweet, 1935) and ablation was verified in the longest fasting animals by complete serial sections of the base of the brain with the intact dura of the sella turcica. The two dogs which fasted for 27 and 55 days showed exceptionally clean-cut operations. Only a few cells of the most posterior portion of the pars tuberalis remained *in situ* and there was no apparent damage to the hypothalamus. In all of the animals the concentration of sugar in the arterial blood, determined at frequent intervals, fell gradually during inanition to a level of about 30 mgm. per cent and usually was maintained there for several days before preliminary symptoms of hypoglycemic shock appeared. The fast then was terminated by giving glucose, and changes in the respiratory metabolism were determined. Four of the dogs needed glucose in 5 to 8 days, four others in 11 to 16 days, but four went without food for 24, 27, 30, and 55 days. No explanation can be offered for this marked variation in the length of time which elapsed before the hypoglycemic symptoms developed. The basal respiratory quotients fell to the usual fasting value of 0.70 to 0.72 by the 5th day. In the shorter fasting groups, 5 to 16 days, the response to ingested glucose was within the normal range or slightly better. The non-protein respiratory quotients were about 0.85, and

glycosuria occurred in only a few instances. When glucose was administered to the three dogs which fasted for 24, 27, and 30 days the results were typical of animals with the pituitary intact. The respiratory quotients did not rise above the basal level of 0.71 for 4 hours, and hyperglycemias from 225 to 315 mgm. per cent were found. Further evidence of a normal reaction was obtained by giving a second dose of glucose to the 24-day fasted dog. The respiratory quotient of 0.83 and hyperglycemia of 215 mgm. per cent are in the range of the unoperated fasted animals shown in table 4 (p. 276). It is doubtful if the premortal stage of fasting can be reached by the hypophysectomized dog. Although the loss in body weight was 43 per cent after 55 days of fast, the nitrogen and creatine excretion did not increase and there was no diminution in urinary acetone bodies. Thus the absence of the hypophysis did not prevent the development of hunger diabetes in the animals which were able to survive the prolonged hypoglycemia, and therefore the anterior lobe does not seem to be directly involved in the impaired carbohydrate metabolism of inanition.

The effect of a high fat diet after extirpation of the pituitary also has been studied. The daily changes in blood sugar were observed in six hypophysectomized dogs when fasted or fed exclusively on fat or on protein by Soskin, Mirsky, Zimmerman, and Crohn (1935). The average daily blood sugar concentration fell below 20 mgm. per cent in 10 days of fat diet or 12 days of fasting in one typical animal and more rapidly, 3 and 5 days, in another. Exogenous fat was therefore just as ineffective as body fat in maintaining the blood sugar at a constant level. The protein diet furnished an adequate supply of glucose. The evidence for the conclusion that this condition is restricted to the hypophysectomized animal is not convincing. High fat and high carbohydrate diets have been compared on the basis of the response to insulin and to the administration of glucose (Weichselbaum, Heinbecker, and Somogyi, 1937; Heinbecker, Somogyi, and Weichselbaum, 1937). In all of the hypophysectomized dogs the high carbohydrate diet improved the tolerance for glucose and increased the reaction to insulin. In the normal controls the effect of the diets was not as consistent as one would expect from the work discussed in an earlier section of this paper (p. 250), perhaps because a generous amount of protein was contained in the fat diet (100 grams fat plus 350 grams raw lean meat).

Recent experiments on the very early stages of fasting in the rat indicate an increased oxidation of carbohydrate at this time if the pituitary gland is absent. This would explain the more rapid loss of

glycogen (Russell, 1936) and the slower fall in basal respiratory quotient which sometimes did not reach 0.73 until between 30 and 40 hours after food (Fisher and Pencharz, 1936). Experiments to determine a balance between the disappearance of muscle glycogen and the oxidation of carbohydrate in the postabsorptive state have been reported by Fisher, Russell, and Cori (1936). The muscle glycogen content was approximately the same in normal rats fasted for 18 hours and in hypophysectomized rats fasted for 10 hours. The average non-protein respiratory quotients were 0.72 and 0.76, respectively, for the next 6 or 7 hours. The loss in muscle glycogen during this period was calculated from the analyses to be 2.7 mgm. per cent per hour in the normal group and 12.2 in the operated group. Thus a greater rate of carbohydrate oxidation was correlated with a more rapid depletion of muscle glycogen. Hypophysectomized rats treated with anterior lobe extract resembled the normals. Whether these reactions can be applied to other species remains to be determined. That the dog may differ from the rat is indicated by the normal basal quotients and the normal response to ingested glucose at 18 hours after food, as well as by the low basal quotients after 5 days of fasting.

Acidosis and ketosis. Impressed with the similarity in the symptoms of experimental hunger diabetes and of malnutrition in infants, Elias and Kolb in 1913 proposed the theory that the accompanying acidosis was responsible for the impaired tolerance. The administration of Na_2CO_3 to dogs undernourished for 1 to 2 weeks apparently reduced the glycosuria and hyperglycemia of the glucose tolerance test coincident with the improvement in blood alkali reserve. However, the control tolerance tests were made before the alkali was given and the effect of repeated doses of glucose was not appreciated. A number of rabbits failed to develop a ketosis or acidosis when fasted for 4 days but responsed favorably to the ingestion of $NaHCO_3$ (du Vigneaud and Karr, 1925). Daily administration was more effective than a single dose. Hirsch (1927) used sodium acetate to improve the tolerance of rabbits and dogs, and likewise in a human subject after a fast of 38 hours. The beneficial effect of alkali administration in man and in the dog has been questioned by several of the more recent workers. No improvement from 10 grams of $NaHCO_3$ was noted by Kageura (1923) in the tolerance of dogs maintained on a meat-fat diet. A human subject receiving a low carbohydrate diet exhibited the same hypersensitivity to sugar when given in addition 12 grams of $NaHCO_3$, despite the compensation of the acidosis (Malmros, 1928). Similar results with

children on a ketogenic diet plus 10 grams of $NaHCO_3$ have been reported by Ellis (1931). Goldblatt and Ellis (1932) found that in a normal man 40 hours after food 10 grams of $NaHCO_3$ in two doses raised the plasma CO_2 over 20 volumes per cent and the alkalinity of the urine from pH 5.0 to pH 8.0 without improving the utilization of ingested glucose. The same effect was seen in dogs without food for 7 days; the daily administration of 1 or 10 grams of $NaHCO_3$ produced no significant difference in the reaction to glucose (Chambers and Marquis, unpublished data).

Another approach has been the production of acidosis by ingestion of NH_4Cl. Amounts sufficient to lower the blood alkali reserve 50 per cent or more have given variable results with the glucose tolerance test. An improvement in one adult was reported by Lennox (1927), but evidence of a diminished response was found by Malmros (1928) and by Thompson, Mitchell, and Kolb (1933). The disturbance in children receiving 5 grams per day was not consistent but was not comparable to that produced by a ketogenic diet (Gilchrist, 1932). No definite change from the normal was noted by Deuel and Gulick (1930) in well-fed dogs. Most of the foregoing investigators agree that the level of alkali reserve or acidosis *per se* is a factor of minor if any importance in the explanation of hunger diabetes.

The presence or production of ketone bodies as a possible factor is not eliminated by the experiments cited above, since correcting the acidosis with $NaHCO_3$ does not reduce the urinary ketone excretion (Malmros, 1928; Goldblatt and Ellis, 1932). Cori and Cori (1927) discovered that rats fasted for 48 hours during the summer months excreted ketones in an amount about three times as great as those fasted in the winter. Glucose tolerance tests showed that glycogen formation was not impaired, but oxidation was reduced about 50 per cent and the analyses could account for only 71 per cent of the injected sugar. The deficiency in oxidation and in recovery of glucose was restored by the administration of insulin. A differential interference with insulin activity by injected acetone bodies has been suggested by Sevringhaus (1926, 1927), and a reduction in glucose tolerance after acetone administration has been reported recently by Kunde, Becker, Saruk, and Kearney (1935).

In the condition of prolonged fasting ketone body excretion does not seem to be related to the ability of the body to oxidize ingested glucose. In the group of dogs which showed the typical premortal increase in nitrogen excretion during the fourth stage of inanition (p. 265) the

excretion of ketones was definitely reduced, in some instances to an undetectable amount. In the other group, which were characterized by a lack of premortal rise in nitrogen excretion, there was no marked change in urinary ketone bodies between the third and fourth stages of fasting. The response to ingested glucose was the same in both groups. Similar results were noted in three depancreatized dogs in the premortal state (Barker, Chambers, and Dann, 1937). Ketonuria disappeared in the animal with an increased protein metabolism, but the other two dogs excreted 26 and 35 mgm. of acetone bodies per hour. A definite rise in respiratory quotient after glucose administration was noted in each case. Thus some oxidation of glucose was found even when the ketone excretion was at the diabetic level.

Dietary factors. Various food materials have been studied in regard to their influence in restoring the carbohydrate metabolism to normal after a period of low carbohydrate diet or inanition. Complete recovery is not attained immediately after changing from a low to a normal carbohydrate diet. It may be several days before the glycosuria disappears (Odin, 1927), and then a period follows in which the glucose tolerance is supernormal (Kohn, Fries, and Felshin, 1927; Ellis, 1931). The high postabsorptive respiratory quotients during realimentation after a fast indicate a greater proportion of energy from carbohydrate than fat (Takahira, 1925; Dann and Chambers, 1933) and after glucose ingestion the quotients show an unusually large formation of fat. The administration of pure glucose alone can restore the tolerance to normal (p. 276 and table 4), but whether or not a complete recovery of oxidation can be obtained by this treatment remains an open question. Non-protein quotients between 0.90 and 0.95 were reached with the third 50 grams dose of glucose, but additional amounts up to 400 grams gave no further significant increase.

In the initial phase of recovery the stimulating effect of a single meal of other foodstuffs has been compared with that of glucose. Since in many cases the food was given 18 hours before the glucose test, a differentiation should be made between the substances containing carbohydrate, either actual or potential, and those supplying no glycogenic material. Protein in the form of gelatin or egg albumin increased the tolerance of rabbits fasted for 4 days when it was ingested 18 hours before the glucose test (du Vigneaud and Karr, 1925; Harding and van Nostrand, 1929) but had little effect when administered with the glucose. These investigators disagree in their data concerning the effect of a single meal of fat, but it seems doubtful if ingested fat and endog-

enous fat differ in this respect. The results of Reinhold and Karr (1927) that fructose, galactose, and maltose were effective restoratives but lactose or starch were not, are more difficult to interpret because the glucose test was performed about 2 hours after the other carbohydrate was given, in most instances before the blood sugar had returned to the basal level.

A similar recovery effect with protein has been obtained in dogs fasted for 3 or 4 weeks (Chambers and Dann, 1931, and unpublished data). Five experiments in which meat, gelatin, or casein was eaten 18 hours before the glucose test, yielded results quite like those with insulin, i.e., non-protein quotients of 0.80 and an average glycosuria of 3.1 grams, but the protein in amounts up to 100 grams was not equivalent in beneficial action to 50 grams of glucose. A recovery diet of meat and glucose was no more effective than glucose alone in restoring carbohydrate utilization. Foods containing polysaccharides—50 grams of cracker meal (wheat flour), 30 grams of maltose, or maltose fortified with wheat germ extract (Vitavose)—all produced a greater improvement than equivalent amounts of glucose. Respiratory quotients for 3 hours after the glucose test averaged between 0.88 and 0.91 and in several instances surpassed 1.00 in the later hours. Glycosuria was slight or absent (Dann and Chambers, 1933).

The stimulating value of the cracker meal and wheat germ extract beyond their carbohydrate content led to the investigation of some other food factors containing little or no carbohydrate. A definite benefit somewhat better than that from protein or insulin was obtained with 5 to 20 grams of dry yeast or a powdered yeast extract rich in vitamin B complex but not with 1 gram of dry yeast. Unlike insulin, 50 grams of yeast were ineffective if eaten 36 hours before the glucose test. Some of the experiments indicated an additive effect of insulin plus yeast, but the data are insufficient for a quantitative treatment. Liebig's beef extract was of no value in stimulating carbohydrate metabolism. The results obtained with wheat embryo, active diastase, or 1 mgm. per kgm. of histamine were in the range with those from insulin or protein (Dann and Chambers, 1933, and unpublished data). Some of these materials may react by stimulating the pancreas (Heller, 1935; Laughton and Macallum, 1935), but the yeast extract exhibited a greater efficiency than large amounts of injected insulin, suggesting an additional factor in this case. It is doubtful if the action of yeast is due to a vitamin B fraction, as Burack and Cowgill (1932) found the impaired glucose tolerance of the polyneuritic dog was correlated with

the degree of inanition rather than with the avitaminosis. It should also be noted that rather large amounts of yeast in the diet have little effect on the glucose excretion of depancreatized dogs (Nasset, Pierce, and Murlin, 1931).

The literature concerning the influence of undernutrition on galactose metabolism has been discussed in a recent review by Deuel (1936). The tolerance for galactose as judged from the blood sugar data is not diminished by fasting according to most of the evidence. The experiments have all been performed in the first stage of inanition. It is possible that this distinction between galactose and glucose will not be found if the fast is extended to the intermediary stages.

THEORIES OF OXIDATION. The well-known theory that glucose must be transformed into glycogen before it can be oxidized has been suggested as the explanation for the delayed reaction in hunger diabetes (Bernstein and Falta, 1918; Goldblatt and Ellis, 1932). Part of the data from the undernourished animals and some of the recent work in pancreatic diabetes lend support to this theory. In a general way the ability to oxidize carbohydrate varied directly with the glycogen content of the tissues. When the predominating foodstuff of the diet was changed from carbohydrate to protein and then to fat, the liver glycogen diminished and also the tolerance for glucose. Similarly, as the period of inanition progressed through the first and second stages, a decrease occurred in both liver and muscle glycogen and in the response to administered glucose. A part of the first glucose meal after fasting was retained in the liver and muscles as glycogen and there was then a marked improvement in the oxidation of the second dose of sugar.

A more striking correlation is seen when comparing the presence or absence of the pituitary gland in the depancreatized dog. Extremely low values for glycogen are found in the completely depancreatized dog, which has been fasted for several days. Reports from several laboratories show a range for both liver and muscle of approximately 50–200 mgm. per cent (Fisher and Lackey, 1925; Major and Mann, 1932; Chambers, Kennard, Pollack, and Dann, 1932; Bodo, CoTui, and Farber, 1933). The evidence for the lack of oxidation of exogenous sugar under such conditions has been recently emphasized according to the criteria of no rise in respiratory quotient, no sparing action on protein metabolism or ketone body excretion, and a quantitative recovery of the glucose in the urine (Barker, Chambers, and Dann, 1937). In contrast are the data from the Houssay dog, without pancreas or hypophysis. The glycogen content of the tissues approaches

the normal state and is increased after glucose administration (Houssay, Biasotti, and Dambrosi, 1936). Animals which have not been fasted show glycogen concentrations of about 2 per cent in the liver and about 500 mgm. per cent in the muscle (Collip, 1935; Houssay, Biasotti, and Dambrosi, 1936; Chaikoff, Gibbs, Holtom, and Reichert, 1936; Shorr, unpublished data; Chandler, unpublished data). The indications for glucose oxidation are seen in the rise in respiratory quotient in some of the animals after glucose ingestion or injection (Houssay and Biasotti, 1931; Biasotti, 1934). The basal quotients are usually higher than 0.72, the diabetic level of the depancreatized dog, and the balance studies indicate that the glucose unrecovered in the urine is oxidized (Chambers, Sweet, and Chandler, 1937). However, both oxidation and glycogen formation are limited to a low level beyond which all of the dietary glucose is excreted. The theory offers an attractive explanation for the delayed effect of insulin on carbohydrate oxidation as noted above (p. 281), since the glycogenic action of insulin in the muscle is well recognized. However, the necessary evidence from the actual glycogen determinations has not been produced in this case.

The correlation between glycogen content and glucose oxidation is not so consistent in some of the other experiments. Data on the premortal condition indicate a very low level of glycogen, particularly in the muscle, perhaps lower in some cases than that of the depancreatized animals cited above. Yet after a prolonged fast the recovered ability to oxidize carbohydrate was shown by the normal respiratory quotients for protein and their elevation following glucose ingestion in both normal and depancreatized animals. Recent experiments with a nephrectomized-depancreatized preparation (Mirsky, Heiman, and Broh-Kahn, 1937) demonstrated a ketolytic effect from intravenous glucose which suggested an association of glycogenesis and glucose oxidation, but determinations of the respiratory metabolism and glycogen content of the tissues indicate that glycogen formation in the absence of insulin does not necessarily restore the oxidative mechanism (Barker and Sweet, 1937). Successive intravenous injections elevated the blood glucose to about 2000 mgm. per cent in 20 hours without significantly changing the respiratory quotient from the diabetic level. At the same time glycogen was being deposited rapidly in the muscle, reaching about 400 mgm. per cent, which is a value only slightly below normal, but at a subnormal rate in the liver where the concentration averaged 800 mgm. per cent at the end of the experiment.

Cardiac muscle offers another example of a disproportion between

glycogen content and carbohydrate oxidation. The increase in cardiac glycogen on removal of the pancreas is well known and is associated with a diminished or a complete absence of response to glucose (Evans, 1936; Himwich, Goldfarb, and Fazekas, 1936; Cruickshank, 1936; Shorr, Malam, and Richardson, 1937). These instances in which evidence of glucose oxidation is found when the glycogen content of the tissue is low and the converse situation, a lack of oxidation with a high glycogen concentration, introduce a serious doubt as to the dependence of oxidation upon the accumulation of glycogen. Various experimental procedures inhibit the rate of glycogenesis from glucose, but none has been shown to abolish the ability to form glycogen. Therefore the possibility of glycogen as an intermediate step in oxidation cannot be ruled out by the present data.

The studies of inanition suggest that carbohydrate oxidation is a fundamental function of the tissue cells which can be suppressed by the diminution of available glucose but which is recovered at least partially after prolonged fasting. Recovery occurs even when there is no elevation of protein catabolism to furnish potential carbohydrate, and conversely the mere accumulation of carbohydrate in the tissues as glycogen does not lead necessarily to its oxidation. The data suggest that some substance other than glycogen, as a preliminary step in oxidation, might explain the accumulated facts. The endocrine secretions influence the process but do not control it completely. Removal of the pancreas, which arrests oxidation in the early fasting stage, does not prevent recovery in the premortal stage. Recent observations on isolated tissue from depancreatized dogs may be significant in this connection (Shorr, 1937). If the tissue is incubated for a sufficient length of time after excision from the body, the ability to respond to glucose is rapidly restored. Ablation of the pituitary gland enables the depancreatized animal to oxidize carbohydrate and deposit glycogen to a limited extent. Evidence for a direct inhibitory action on glucose oxidation by the anterior lobe of the pituitary (Marks, 1936) is not supported by the studies of the intermediate stages of fasting in the hypophysectomized animal with the pancreas intact. Attenuation of pancreatic diabetes by adrenalectomy (Long, 1937; Houssay, 1937) indicates that the adrenal cortex may influence carbohydrate oxidation.[2]

[2] Recent respiratory metabolism data have provided additional evidence for this effect of the adrenal. An elevation of the respiratory quotient above the diabetic level has been reported in depancreatized animals with the lumboadrenal veins ligated (Fazekas, Himwich, and Martin. Science **87**: 144, 1938). Four adrenalectomized-depancreatized dogs have been observed in this laboratory

The influence of thyroidectomy in the fasting organism appears to be a minor one. Thus the experimental work in undernutrition discussed in this review has modified our conceptions of the conditions regulating carbohydrate oxidation in the intact organism and introduced new facts which must be considered in the formulation of any adequate theory of control. These studies also emphasize the importance of the dietary supply of carbohydrate in the treatment of diseases involving deficient carbohydrate metabolism.

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PRESENT STATUS OF THE ERGOT QUESTION

ERWIN E. NELSON AND HERBERT O. CALVERY

Department of Pharmacology, Tulane University of Louisiana Medical School, and the Division of Pharmacology, Food and Drug Administration, United States Department of Agriculture

The masterly review of *Ergot and Ergotism* by Barger (2) makes unnecessary any discussion of the literature prior to that date. In retrospect it seems that this review was prepared at the opportune time. With the isolation of ergotamine and its isomer ergotaminine, and the extensive pharmacological studies made with ergotamine and with the crystalline preparations of ergotoxine which had recently been made available, it seemed that a somewhat stable period had been reached. The view was held in many places that the then known alkaloids were responsible for the clinical actions of ergot. Assay methods, both chemical and biological, which attempted to measure the alkaloidal content were adopted in many of the Pharmacopoeias. In the United States the Council on Pharmacy and Chemistry of the American Medical Association refused recognition to certain non-official preparations on the ground that they contained no alkaloids (10). An extensive clinical literature was developed on the value particularly of ergotamine. Although the view generally prevailed that ergotoxine and ergotamine were essentially equivalent in their pharmacological action strangely enough ergotoxine did not seem to meet clinical needs. According to Dale this may have been due to its never having been given a fair trial (15). It is possible that the fact that ergotoxine was a non-proprietary product may have been a factor as well.

Although one finds here and there some suggestion of reservation regarding the view that the action of ergot was represented in these alkaloids, the first real dissent came with the development of observations by Chassar Moir (60) in 1932 that the British Liquid Extract of Ergot, and the solid extract as well, both of which had been shown to contain very little of the ergotoxine-ergotamine group of alkaloids, were markedly and promptly stimulant to the human puerperal uterus, when the preparations were given by mouth. Moir showed as well that the action differed from that following the administration of the

purified alkaloids. In commenting on this paper Dale indicated that these observations might well be the opening of a new chapter in the history of ergot (16). He also made the statement that Dudley had made some progress in identifying "the type of the substance in ergot responsible for this prompt action with oral administration."[1]

In 1932 a new alkaloid, sensibamine, was reported by Wolf (9), and in 1933 another, ergoclavine, was isolated by Küssner (56). These substances apparently are pharmacologically similar to the ergotoxine-ergotamine group, and are therefore not responsible for the type of action described by Moir.

In September 1934, Swanson and Hargreaves (92) reported the results of the examination of the action of ergot and its alkaloids on the puerperal uterus of dogs by a technic similar to that used by Moir in his clinical study. A balloon was inserted into the intact uterus of the dog, three to six days post-partum. Contractions of the uterus upon this balloon were recorded graphically. As a result of the administration of a number of ergot preparations by various routes these authors reached the conclusions that "On the puerperal uterus the fluid extract of ergot U.S.P., liquid extract of ergot B. P. 1914 and the various extracts by mouth show a more rapid onset of definite contractions than that of the alkaloids, ergotamine or ergotoxine. Ergot contains a principle (not ergotamine or ergotoxine) that produces by mouth a rapid onset of action on the puerperal uterus of dogs."

In January and March 1935 there appeared the report of an ex-

[1] As will appear, the question of priority of discovery and the naming of certain new compounds derived from ergot has caused much discussion. The writers of this review have attempted to give a summary of the various *published* reports in chronological sequence so far as possible without attempting to settle controversial issues. Since five names have been suggested for the substance the history of whose discovery is delineated in the next few paragraphs, it will perhaps best serve the reader to introduce the terms at this point. The names, obviously synonyms, are ergotocin, ergometrine, ergobasine, ergostetrine, ergonovine. In each case the name used in the present section of the review will be that used by the author or authors of the article under discussion. To be consistent however the termination "ine" rather than "in" will be used uniformly except in the case of ergotocin, because of the insistence of those who first used the name that the substance it represented was not an alkaloid.

So far as possible, the various reports will be discussed in the sequence in which they were published rather than according to the dates of public presentation. This is the more necessary because such presentation is usually of much condensed material, and stenographic reports of the papers and of the subsequent discussion are seldom available.

tensive study by Thompson (94) in which he undertook to determine whether the oxytocic effects of the purified alkaloids (ergotoxine and ergotamine) were the same as the effects produced by the crude extracts when administered to intact pregnant cats. In the first part of this study (January) he states: "It may be concluded therefore that Moir's and Dale's belief that a highly important unidentified substance exists in ergot and its crude extracts (both aqueous and alcoholic), is confirmed by these studies. Quite contrary to their apparent belief, however, this substance is shown to behave characteristically as an alkaloid, since the studies here reported show the presence of this new substance in the completely colorless "Total Alkaloidal Fraction," while the "Alkaloid-free Fraction" proved to be devoid of any oral or subcutaneous activity of any significant type. The writer therefore believes that this new substance must be classified chemically as a new member of the "total specific alkaloids" of ergot, and that the activity of aqueous extracts, observed by Moir in human patients, is to be ascribed to 'residual alkaloid'."

In February 1935 Koff (54) reported clinical studies using the method of recording the contractions of the puerperal uterus described by Bourne and Burn (5) and used by Moir (60) and in part administering ergot preparations supplied to him by Thompson, to whom he makes acknowledgment. He obtained results similar to those of the above mentioned workers, regarding the slowness of onset of action of the ergotoxine-ergotamine group of alkaloids, even when given intramuscularly, and emphasized the more prompt action of oral administration of the fluidextract. What is of greater significance, he found that the alkaloid-free preparations supplied him by Thompson were without oxytocic action when given by mouth in large doses, whereas ergot preparations containing only the total alkaloids exerted "an oxytocic effect similar to the U.S.P. fluid extract of ergot, and to aqueous extract. This indicates that the active principle is either an alkaloid, or some chemical substance closely associated with the alkaloidal fraction."

In February 1935 there also appeared a paper by Davis, Adair, Rogers, Kharasch, and Legault, on "a new active principle in ergot, and its effect on uterine motility" (21). The following paragraph is quoted from this report: "Moir, in 1932 while studying the B.P. aqueous extract of ergot, noted that although it was low in alkaloidal activity it proved to be high in physiologic activity when tested on the human uterus. Thus for the first time it was suggested that all the

activity present in ergot might not reside in its alkaloids. Since we had been working on the same subject, independently, Moir's publication again focused our attention on the ergot problem."

In the paper there was described a new principle, in the *non-alkaloidal* fraction of ergot, active in 3 mgm. doses, given orally. Studies of the purification and determination of the exact chemical nature were said to be under way. In a footnote added after presentation of the paper, it was stated that the "pure, active principle in crystalline form" had been isolated and found therapeutically active in doses of 0.1 mgm. Another footnote indicated that it was to be made available in large quantities for clinical assay by the Eli Lilly Company under the trade name "Ergotocin."

The second part of Thompson's paper referred to above appeared in March (95) and contained the statement that he had separated an alkaloid in sufficiently pure amorphous condition to permit certain pharmacological and chemical comparisons with the hitherto known alkaloids. The differences lay chiefly in the direction of a greater solubility in water and a more prompt and powerful oxytocic activity after oral administration. The term X-alkaloid was applied to this substance. In a footnote, Thompson added that the amorphous X-alkaloid had yielded a crystalline product.

In the British Medical Journal for March 16, 1935, Dudley and Moir reported the isolation of a new substance "responsible for the traditional clinical effect of ergot" and proposed that it be called "Ergometrine" (23). Certain details of the method of isolation, solubilities, and analyses of the material crystallized from benzene were given, with the reservation that modifications might be necessary when larger amounts were available for analysis. It was believed that the new substance was an alkaloid, probably simpler than any which had hitherto been isolated from ergot. Clinically in doses of from 0.5 to 1.0 mgm. (orally) it provoked marked contractions of the uterus similar to those which had been reported by Moir for the British liquid extract. By intramuscular injection 0.25 and 0.5 mgm. were effective, and in two cases 0.05 and 0.1 mgm. given intravenously were followed by prompt contractions, in each case in less than two minutes. In this paper the authors report briefly the findings of Dale and Brown with respect to vasomotor reversal in the cat, and the abolition of the stimulant effect of epinephrine on the isolated rabbit's uterus, namely, that the actions are so weak in comparison to those of ergotoxine that they might well be due to slight contamination with this substance. Ref-

erence is made to the work of Koff. A brief review of the paper of Dudley and Moir was printed in Science for March 29, 1935 (24).

On April 19, 1935, appeared a note by Kharasch and Legault (51) in which it was stated that isolation of a pure crystalline principle had been made December 12, 1934. This principle was to be called "Ergotocin" although the name had already been applied to the partially purified product mentioned in the earlier paper (21). Both salts and base were described as white crystalline materials: the salts so far as examined were dextro-rotatory. Melting points of base and picrate were given. The base was said to be somewhat soluble in water, the salts readily soluble.

The May issue of the Comptes rendus des séances de l'Académie des Sciences contained a report by Stoll and Burckhardt (88), to the effect that they had isolated a new water-soluble alkaloid of ergot to which they gave the name "Ergobasine." Because of differences in the properties reported for ergometrine by Dudley and Moir, and those which they described for ergobasine, Stoll and Burckhardt believed that the two substances were not the same. In a second report, also published in May (89) extensive chemical details, melting points, solubilities, optical rotations, details of the method of isolation were given. An empirical formula, $C_{19}H_{23}O_2N_3$, which has been confirmed by Jacobs and Craig (38), was given. The name suggested was proposed because of the strongly basic properties.

In Science for June 7, 1935, appeared a note from Dudley and Moir (25) acknowledging that work had been progressing simultaneously with theirs in two laboratories in the United States, and that isolation of the substance and publication of the findings had been practically simultaneous. In the Pharmaceutical Journal for June 15, 1935 (22), Dudley published a note on the preparation of ergometrine, and gave the melting point as 161°–162°C. In the June issue of the Journal of the American Chemical Society (52) Kharasch and Legault gave analyses to support an empirical formula, $C_{21}H_{27}O_3N_3$, and described certain of the chemical properties of ergotocin. At that time they declined (53) to accept the suggestion of Dudley and Moir that ergotocin and ergometrine were one and the same substance (25), because of differences in analytical results, and because their preparation was active in doses smaller than those found by Dudley and Moir. Thompson however, in spite of the differences found by the different investigators insisted that ergotocin and ergometrine were the same as his X-alkaloid, which he now stated had been given the name Ergostetrine in an application for a patent in May, 1934 (97).

The following month Jacobs and Craig (38) reported that they had isolated an alkaloid which possessed the properties described for ergobasine by Stoll and Burckhardt, and which they believed to be the same material. To this substance they gave the empirical formula $C_{19}H_{23}O_2N_3$.

The history of these developments was reviewed by Dale (17) who put forth the suggestion that the four names, ergobasine, ergotocin, ergostetrine, and ergometrine, were different names for the same substance. Subsequently there appeared a note signed by Kharasch, King, Stoll, and Thompson, indicating their accord that the alkaloid obtained in the four different laboratories was one substance. "Having reached this conclusion, we are content to leave to the world of science the choice of one of these names, for adoption into scientific literature as the recognized name of the alkaloid" (50).[2]

In May, 1936, the Council on Pharmacy and Chemistry of the American Medical Association authorized the publication of a notice which read in part as follows: "It is necessary.... that a suitable non-proprietary name which is not therapeutically suggestive be adopted for the new alkaloid. Not one of the several names that have been proposed by the discoverers complies with these requirements....." "The Council....therefore determined to adopt the new, non-proprietary name 'Ergonovine.' .... It seems impossible to establish undisputed priority. The Council has therefore decided it would recognize no proprietary name" (11).[3]

Subsequently Smith and Timmis have reported the isolation of an isomer of ergometrine, ergometrinine (82), as well as another pair of alkaloids, ergosine and ergosinine (81). A second isomer of ergotinine, psi-ergotinine, which is distinguished from ergotinine only by its greater specific rotation and greater solubility was described by them in 1931 (76).

The British Pharmacopoeia has adopted the term ergometrine. Those who are concerned with the sale of the alkaloid in the United States have accepted ergonovine as a synonym. In order to avoid awkwardness and circumlocution, the present reviewers have used the term ergonovine throughout the balance of this paper, except when specific reference to work of the British group is made.

Chemistry of the ergot alkaloids. The very extensive and complete discussion of the chemistry of ergot by Barger in chapter IV of his

[2] Doctor King acted in place of the late Dr. H. W. Dudley, who died Oct. 3, 1935.

[3] See the report of the Council on Pharmacy and Chemistry (12) for correspondence between Doctor Dale and the Council concerning the matter of terminology.

monograph makes unnecessary any review of the chemical studies prior to 1931. More recently both Barger (3) and Stoll (87) have presented excellent reviews of the subject.

In table 1 are given the alkaloids at present recognized, together with some of their principal physical properties. These alkaloids, which either exist in ergot, or are obtained by isomerization of the naturally occurring constituents, are: ergotinine and ergotoxine, ergotamine and ergotaminine, sensibamine (which Stoll regards as a crystalline combination of ergotamine with its isomerization product ergotaminine (87)), ergoclavine (which Barger suggests is probably an equimolecular mixture of ergosine and ergosinine (3)), ergometrinine and its isomer (variously called ergometrine, ergobasine, ergotocin, ergostetrine, and ergonovine), and the recently isolated pairs, ergosine and ergosinine, ergocristine and ergocristinine.

Ergomonamine, whose isolation has been reported by Holden and Diver (28), has not been included in the table, since data are not as yet available. Each of those given in table 1 has been crystallized.

In the general use of the term, these substances are all alkaloids. They are soluble in most organic solvents but only sparingly soluble or insoluble in water, with the exception of ergometrinine and its isomer, which can be separated from the other alkaloids by their much greater solubility in water.

The most extensive studies of the composition of the alkaloids are those of Jacobs and Craig (29–48) and of Smith and Timmis (75–84).

Jacobs and Craig have made a planned attack on the ergot alkaloids, with the object in view of determining not only their composition but their constitution. The attack has been made from the following angles: the identification of the products obtained by oxidation with nitric acid; the study of the products obtained by treatment of the original alkaloid with alcoholic potassium hydroxide and aqueous potassium hydroxide; the investigation of the products resulting from the treatment of the alkaloid with sodium and butyl or amyl alcohol; the characterization of the products obtained by strong acid hydrolysis. In their earlier investigations, they were first able to isolate compounds obtained after nitric acid treatment of ergotinine. Among these compounds were nitrobenzoic acid, paranitrobenzoic acid, benzoic acid, and a complex compound containing a methylated nitrogen, three carboxyl groups, and apparently an indole nucleus. Some of these had previously been isolated by Soltys (85) and others (2, 75–84). Following the treatment of ergotinine with alcoholic and aqueous potassium hydroxide, they isolated a compound having the formula $C_{16}H_{16}N_2O_2$ (which they have called lysergic acid), ammonia, isobutyryl formic acid,

TABLE 1

SUBSTANCE	SOLUBILITY*	SPECIFIC ROTATION IN CHLOROFORM	CRYSTALLINE FORM	MELTING POINT
Ergotinine $C_{35}H_{39}N_5O_5$	Water − Organic solvents + Petroleum ether −	$(\alpha)\ ^{19}_{5461} = +513$ $(\alpha)\ ^{19}_{D} = +435$ C = 1%	Long needles, sides not parallel; end symmetrically replaced by a pair of faces, and the extinction is straight	239°
Ergotoxine $C_{35}H_{39}N_5O_5$	Water − Organic solvents + Petroleum ether −	$(\alpha)\ ^{19}_{5461} = -226$ $(\alpha)\ ^{19}_{D} = -197$ C = 1%	Six-sided prisms from benzene	190°–200°
Ergotamine $C_{33}H_{35}N_5O_5$	Water − Organic solvents + Petroleum ether −	$(\alpha)\ ^{20}_{5461} = -181$ $(\alpha)\ ^{20}_{D} = -155$ C = 0.6%	Rectangular plates (aqueous acetone) Long prisms (benzene)	214° (Cor.)
Ergotaminine $C_{33}H_{35}N_5O_5$	Water − Organic solvents + Petroleum ether −	$(\alpha)\ ^{19}_{5461} = +450$ $(\alpha)\ ^{20}_{D} = +385$ C = 0.6%	Plates (alcohol)	252° (Cor.)
Ergometrine (Ergonovine) $C_{19}H_{23}N_3O_2$	Water + Organic solvents + Petroleum ether −	$(\alpha)\ ^{25}_{D} = -44$ $(\alpha)\ ^{25}_{D} = +90$ (in water) C = 0.08%	Needles	158°–160°
Ergometrinine $C_{19}H_{23}N_3O_2$	Water +	$(\alpha)\ ^{20}_{5461} = +520$ C = 1%	Needles	195°
Ergosine $C_{30}H_{37}N_5O_5$	Water − Organic solvents + Petroleum ether −	$(\alpha)\ ^{20}_{5461} = -194$ C = 1%	Needles	228°
Ergosinine $C_{30}H_{37}N_5O_5$	Water − Organic solvents + Petroleum ether −	$(\alpha)\ ^{20}_{5461} = +520$ $(\alpha)\ ^{20}_{D} = +420$ C = 1%	Needles (methyl alcohol) Prisms (alcohol, benzene, ethyl acetate)	228°
Ergocristine $C_{35}H_{39}N_5O_5$	Water − Organic solvents + Petroleum ether −	$(\alpha)\ ^{26}_{5461} = -206$ $(\alpha)\ ^{20}_{D} = -174$ C = 0.5%	Many sided tectiform prisms (acetone)	155°–157°
Ergocristinine $C_{35}H_{39}N_5O_5$	Water − Organic solvents + Petroleum ether −	$(\alpha)\ ^{20}_{5461} = +460$ $(\alpha)\ ^{20}_{D} = +366$ C = 0.675%	Long needles (methyl alcohol) Prisms (methyl acetate)	214°

* All the ergot alkaloids are soluble in organic solvents, except petroleum ether, in which they are not soluble, and insoluble in water, with the exception of the two alkaloids, ergometrine (ergonovine) and ergometrinine.

and in subsequent investigations l-phenylalanine and d-proline. The amide of lysergic acid is identical with ergine reported by Smith and Timmis (79, 81). It hardly seems necessary in this review to recount all the products reported by them before the final hydrolysis products were identified. The ammonia was easy to identify, the lysergic acid could be obtained in pure crystalline form with constant chemical composition, and the isobutyryl formic acid, also previously isolated (2), was identified as a hydrazone, as well as by reduction to alphahydroxyisovaleric acid. The phenylalanine was identified by its physical and chemical properties, and by the identification of the alcohol obtained on reduction of it. The proline was identified by a color reaction for the pyrrole nucleus and by the formation of a gold salt, as well as by the formation of the methyl ester and comparison with the methyl ester of a sample of proline which they had in their possession. This was the first instance reported in the literature of a naturally occurring d-proline, and because of the uniqueness of this finding, in one of their more recent publications, Jacobs and Craig have again prepared the proline from ergotinine and further identified it by the comparison of its specific rotation with the specific rotation of the l-derivative, which is the common form occurring in nature.

The hydrolysis products of ergotoxine are identical with the hydrolysis products of ergotinine. Jacobs and Craig (45), because of the difficulty of elementary analysis of products of such high molecular weight, and because of the variations in the elementary analyses reported by different authors, concluded in one of their publications that the difference between ergotoxine and ergotinine lies in a rearrangement within the lysergic acid part of the molecule, but in their most recent publication they have evidence which indicates that there may be a difference in the actual structure between ergotinine and ergotoxine, in that in the one there is a closed ring which is open in the other. This point is now under further investigation by them. Since the evidence also points to the identity in chemical composition of the two members of each of the pairs of alkaloids mentioned above, the view of Jacobs and Craig that the differences in physical and physiological properties are due to differences in arrangement in the lysergic acid part of the molecule seems reasonable.

By similar lines of investigation to that applied to the identification of the products of hydrolysis of ergotoxine and ergotinine, the hydrolysis products of ergotamine and ergotaminine have been identified, as well as the hydrolysis products of ergobasine and ergoclavine. The ultimate hydrolysis products of ergotamine and ergotaminine are lysergic acid, ammonia, pyruvic acid, l-phenylalanine, and d-pro-

line, as shown in table 2. These two substances differ in chemical composition from the two previously discussed alkaloids by a C_2H_4. This difference is accounted for by the difference between the isobutyryl formic acid and the pyruvic acid. The hydrolysis products of ergometrine are lysergic acid and hydroxyisopropylamine. The pyruvic acid obtained from ergotamine was identified by its phenylhydrazone, and the hydroxyisopropylamine of ergobasine was identified by comparison with a synthetic product of the same structure, as well as by the preparation of the diparabrombenzoate. During their chemical studies, Jacobs and Craig have also studied the hydrolysis products of the ergoclavine reported by Küssner (56), which has more recently been shown by Smith and Timmis (84) to be a mixture of the two alkaloids, ergosine and ergosinine. Jacobs and Craig have found that this is undoubtedly an alkaloid distinctly different from those already men-

TABLE 2*

	ERGOTOXINE ERGOTININE	ERGOTAMINE ERGOTAMININE	ERGOSINE ERGOSININE	ERGOMETRINE ERGOMETRININE	ERGOCRISTINE ERGOCRISTININE
Lysergic acid	+	+	+	+	+
Ammonia	+	+	+	−	
Hydroxyisopropylamine	−	−	−	+	
d-Proline	+	+	+	−	
l-Phenylalanine	+	+	−	−	
l-Leucine	−	−	+	−	
Dimethylpyruvic acid	+	−	−	−	
Pyruvic acid	−	+	+	−	

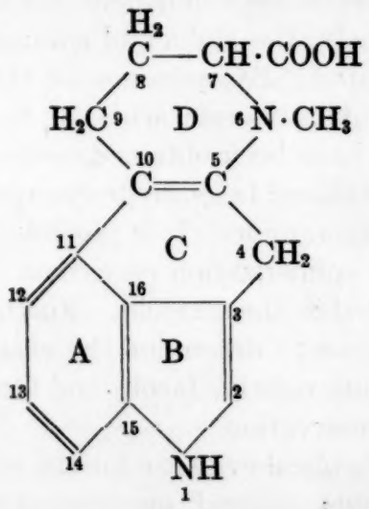
* Partly after Barger (3).

tioned. They obtained as hydrolysis products lysergic acid, pyruvic acid, ammonia, and leucine, only, with traces of proline, isobutyryl formic acid and phenylalanine. They did not believe that these latter substances were hydrolysis products of ergoclavine but that they were obtained from ergotinine, with which their sample of ergoclavine was possibly contaminated. After the identification of ergosine and ergosinine as constituents of ergoclavine, Smith and Timmis were able to isolate from ergosinine both l-leucine and d-proline, so that neither the formula suggested by Küssner ($C_{31}H_{37}N_5O_5$) nor that suggested by Jacobs and Craig ($C_{25}H_{30}N_4O_4$) is correct, but more probably the formula $C_{30}H_{37}N_5O_5$, as suggested by Smith and Timmis. That this is probably accepted by Jacobs and Craig (48) is indicated in their most recent publication, on page 422. The hydrolysis products of ergoclavine (ergosine and ergosinine) are, according to the results of the

above two groups of investigators, lysergic acid, ammonia, pyruvic acid, l-leucine, and d-proline. By condensation of these substances, with the loss of four molecules of water, the above empirical formula ($C_{30}H_{37}N_5O_5$) is obtained.

The characteristic of all of the ergot alkaloids is the presence in the molecule of lysergic acid, and Jacobs and Craig have gone so far as to state that "Lysergic acid is unquestionably the component of the ergot alkaloids to which they owe their pharmacodynamic action." Barger (3) also believes that the remarkable difference in pharmacological properties, optical rotation, and power of associating with solvents result from isomerism in the lysergic acid portion of the molecule. He also believes that the residual valency which the active series evidently possesses is, perhaps, also responsible for the pharmacological action by anchoring the alkaloids to the cells on which they act.

Although lysergic acid can be obtained in relatively large quantities in pure, crystalline form from any or all of the individual alkaloids, the determination of the structure has not been easy, and, as a matter of fact, the final structure of the molecule is not known. The most recent formula proposed by Jacobs and Craig is given below and seems to account for all of the known properties and reactions of lysergic acid.



For example, the indole nucleus is present, which is responsible for the Keller and van Urk reactions; a double bond is present in the molecule, which can easily be reduced to the dihydro derivative; methylated nitrogen is present; and a carboxyl group is present. Evidence for the position of the carboxyl group is still lacking, although it appears to be restricted definitely to ring C or ring D at position (7). Position (4) for the carboxyl group appears less likely, because of the stability of the dihydrolysergic acid. With the carboxyl group at position (7),

the configuration at that carbon could be maintained constant throughout and need play no rôle in the isomerization of the two series of alkaloids. "The isomerism could be due (a) to epimerization on carbon atom (10) caused by shift of the double bond from between (4) and (5) to between (5) and (10) and back again to (4) and (5), or it could be caused (b) by shift so that the double bond is fixed in one series between positions (4) and (5) and in the other between (5) and (10) (or (9) and (10)). If it is assumed that the first of these possibilities, (a), occurs with epimerization on carbon atom (10) with the double bond between (4) and (5), then the following isomers would be expected on hydrogenation. With the fixed configuration on carbon atom (7) of d^7 throughout these would be in the case of one series of alkaloids

$$d^7, d^{10}, d^5, \text{ and } d^7, d^{10}, 1^5$$

and in the case of the other series

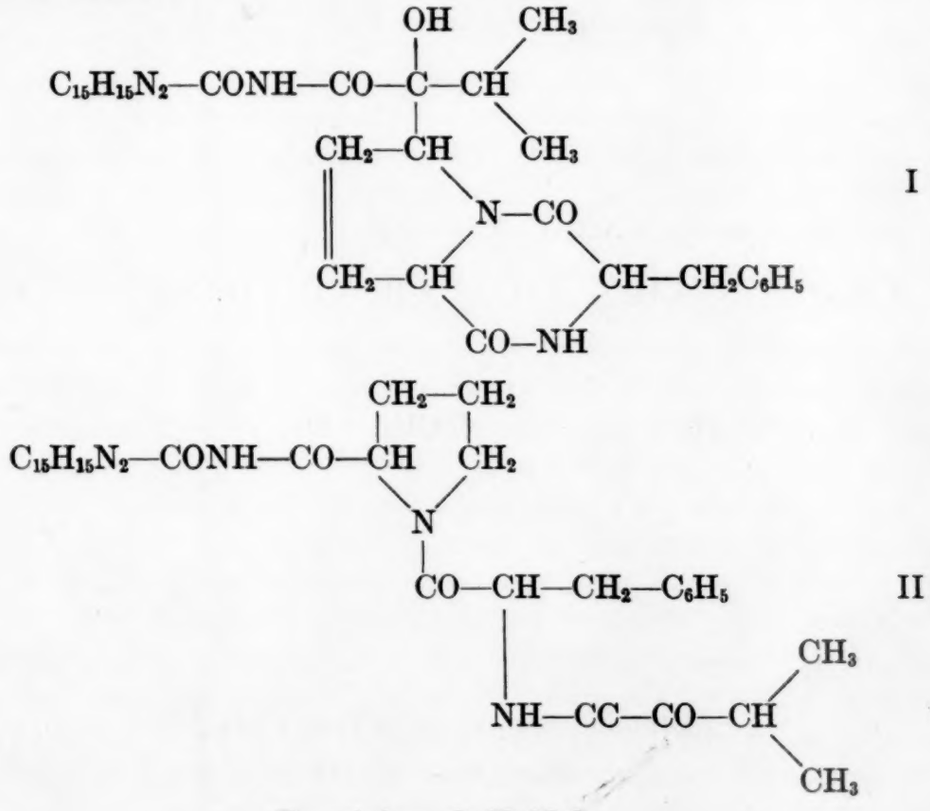
$$d^7, 1^{10}, d^5, \text{ and } d^7, 1^{10}, 1^5.$$

Thus, opportunity would be given for the production of different dihydrolysergic acids from each series of alkaloids." This possibility of isomerization by epimerization can explain the occurrence of two dihydrolysergic acids, namely, the alpha and gamma dihydrolysergic acids, which have been isolated. By reduction of the methyl esters of the alpha and gamma dihydrolysergic acids, alpha and gamma dihydrolysergol, respectively, have been obtained, neither of which is identical with the previously obtained betadihydrolysergol by sodium and butyl alcohol reduction of ergotamine. It is possible that this alcohol could have had its origin in epimerization on carbon atom (7) while lysergic acid was still conjugated in the alkaloid. Further investigations, however, must be carried out to determine the exact relationship of these isomeric alcohols. Quite rightly, Jacobs and Craig have advanced this formula with proper reservation.

In addition to the chemical evidence for the structure of lysergic acid and the ergot alkaloids, several investigators have made spectroscopic analyses and studied the ultraviolet absorption spectra in relation to some other known compounds such as harmine and yohimbine, but the evidence obtained by this means has not aided materially in establishing the formula, other than to show that there is present in each of these alkaloids a similar nucleus, which nucleus undoubtedly is the indole ring. Formulae have been suggested for the alkaloids whose

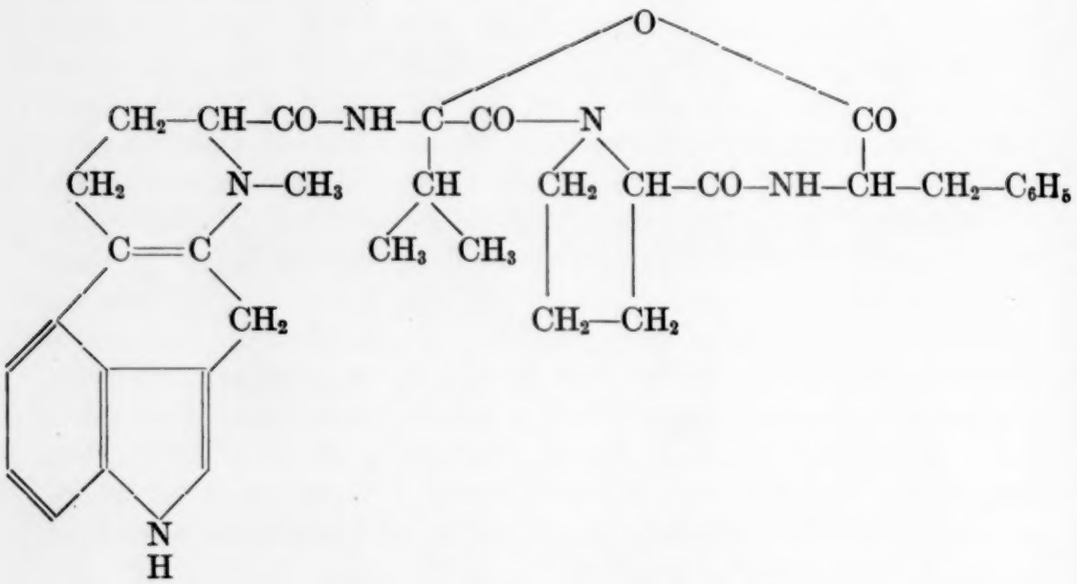
hydrolysis products are known by Turner (98), by Jacobs and Craig (48), and by the authors, and are presented in this review purely for the purpose of stimulation of interest in these products and should not be considered as final. The one best established is that of ergobasinine, since its synthesis has been reported by Stoll (89), but Stoll (personal communication) has emphasized that he does not feel that formulae should be presented, since the formula for lysergic acid is not known.

Concerning the arrangement of the fission products, we may quote from Barger: "The exact arrangement of the various fission products is not known. The lysergic acid is certainly joined to the ammonia (because of the formation of ergine); the isobutyryl formic acid is also joined by its carboxyl to an amino group, for, on pyrolysis, isobutyryl formamide sublimes (Barger and Ewins, 6). The nitrogen atom in the latter amide need not, however, be that giving rise to ammonia, but may be the nitrogen atom of phenylalanine; the two amino acids are also joined together, for their dipeptide has been isolated as a result of moderate hydrolysis."

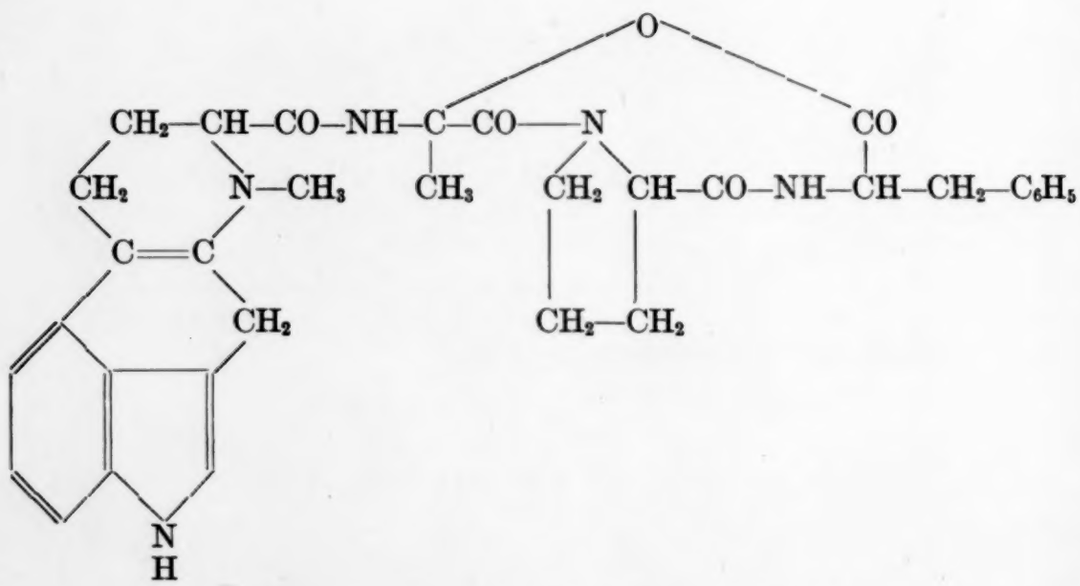


Ergotinine, $C_{35}H_{39}N_5O_5$

Turner (98)

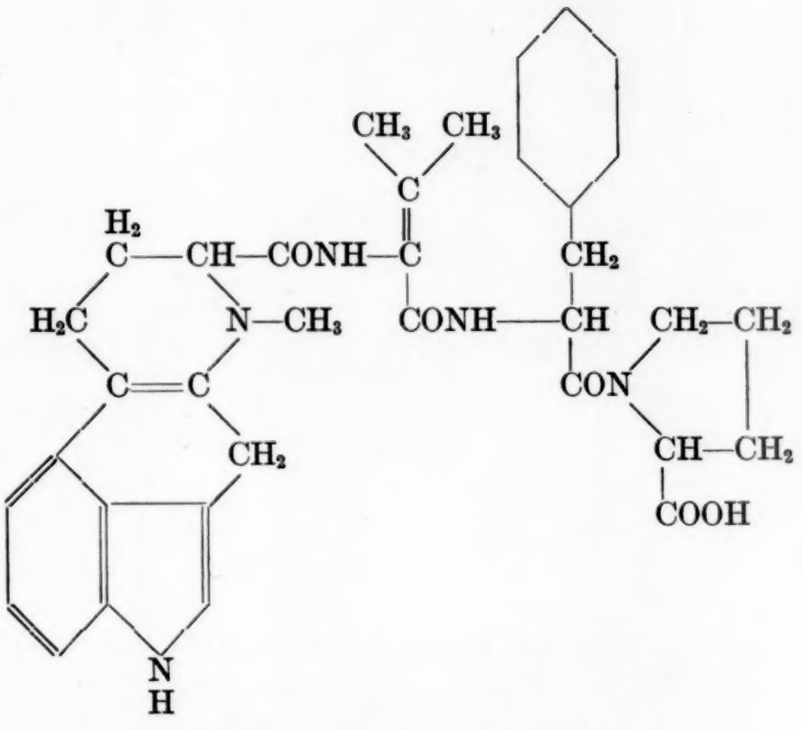


Ergotinine-Ergotoxine Pair, $C_{35}H_{39}N_5O_5$

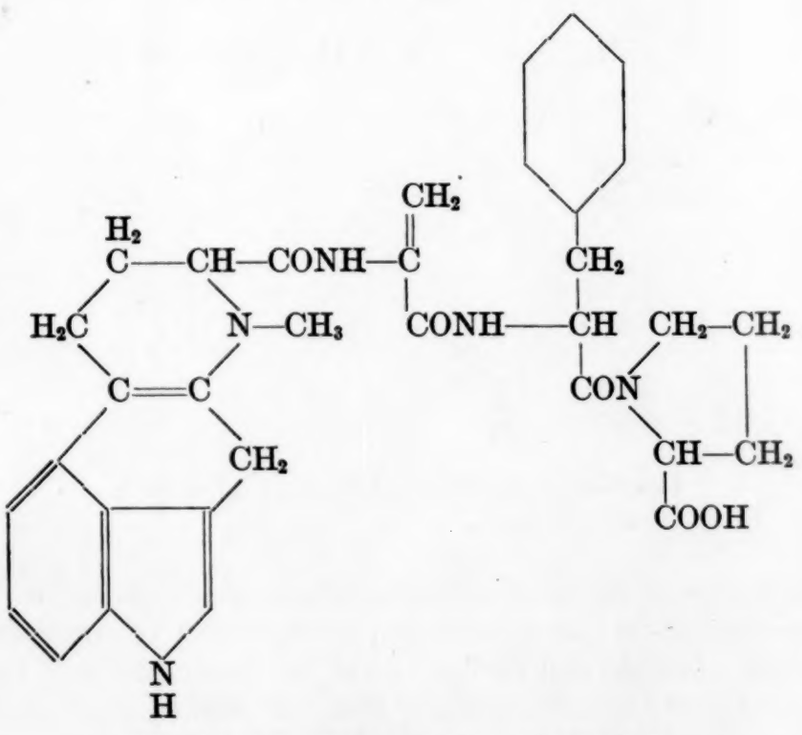


Ergotamine-Ergotaminine Pair, $C_{33}H_{35}N_5O_5$

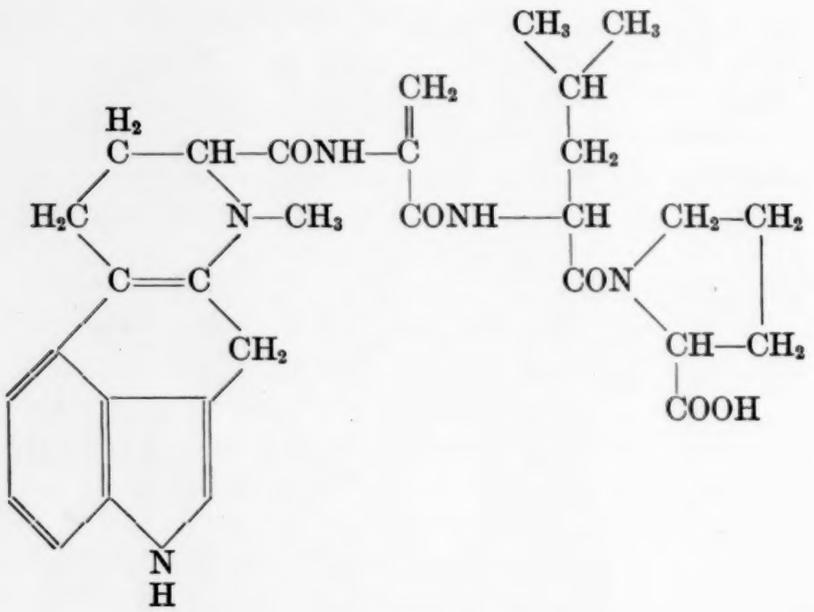
Jacobs and Craig (48)



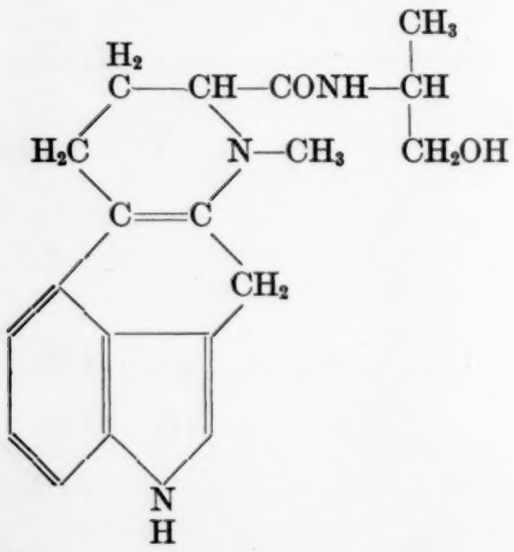
Ergotinine, Ergotoxine, $C_{35}H_{39}N_5O_5$



Ergotamine, Ergotaminine, $C_{33}H_{35}N_5O_5$



Ergosine, Ergosinine, $C_{30}H_{37}N_5O_5$



Ergometrine, Ergometrinine, $C_{19}H_{23}N_3O_2$
(Ergonovine)

Pharmacology of the newer alkaloids, Ergonovine (ergometrine). The pharmacology of this alkaloid is simpler than that of ergotoxine and ergotamine. In 1906 and 1907 in two of the classic papers of Pharmacology, Dale (14) and Barger and Dale (4) showed that ergotoxine caused a primary stimulation of smooth muscle, followed by a selective

paralysis of the motor division of the so-called true sympathetics, or the thoraco-lumbar division of the autonomic nervous system. Ergonovine lacks the action on the sympathetic nervous terminations almost completely. It differs from ergotoxine and ergotamine chiefly in this lack, and in the ease of absorption, especially from the gastro-intestinal tract, and the rapidity of onset of action, especially that on the uterus.

General effects of ergonovine. In the human the only effect from small amounts seems to be the stimulant action on the uterus (1, 59, 26). Adair *et al.* found no significant effect on respiration, pulse rate, blood pressure, or urine output. Moir however has reported three cases in which untoward symptoms occurred after 0.5 mgm. of ergometrine, and raises the question as to whether idiosyncrasy may occasionally exist to this drug (63).

In dogs amounts of ergometrine that were diuretic when given intramuscularly had no other effect (102). Davis *et al.* (20) gave daily, 0.5 and 1.0 mgm. respectively of ergotocin to two dogs, intravenously, for a period of 20 days. The larger dose was followed by a transient albuminuria. No other signs of abnormality were reported. Brown and Dale (7) reported one experiment on a dog, which received a single dose of about 0.8 mgm. per kilo intravenously. There followed a short period of excitability, vomiting, and weakness, especially of the hind legs.

In rabbits, the symptoms are those which follow the other alkaloids in toxic doses, dilatation of the pupil, exophthalmos, marked constriction of the ear vessels, excitement, trembling, marked hyperpnea, and a rise in body temperature. The picture is said to resemble somewhat that produced by other central stimulants, such as beta-tetrahydronaphthylamine (7).

In cats the effect is much more striking. Brown and Dale have described in detail the picture following oral, hypodermic, and intravenous administration (7). Oral administration of 5 mgm. was followed by dilatation of the pupils, retraction of the nictitating membrane, and slight exophthalmos. Salivation may have been due to the bitter taste. Vomiting occurred after large oral doses, and also after hypodermic but not after intravenous administration.

Except for the effect on vomiting, and the differences in rate of onset, the intravenous and hypodermic administrations were followed by essentially the same picture. After intravenous administration, the first actions, which appear very promptly, are similar to those de-

scribed earlier for ergotoxine (4, 18). The pupils dilate widely, exophthalmos is marked, there is erection of the hairs, a marked excitability, and "sham rage." There is definite muscular weakness, even to the point of interfering with locomotion. This weakness however is not so marked nor so persistent as that following ergotoxine. The mydriasis is more marked than that which follows ergotoxine, but does not give place later to miosis, as does the ergotoxine mydriasis (7, 72). Brown and Dale have adduced some evidence to show that this action of ergometrine is not primarily due to a peripheral sympathomimetic effect. When adrenal activity is eliminated, either by section of both splanchnics, or the section of one and the removal of the opposite adrenal, the dilatation of the pupil (previously sensitized by section of the cervical sympathetic) is slower and less marked after ergometrine. This is interpreted as meaning that the adrenal action is central, and that the drug itself has at most a minor part in producing dilatation by direct action. Rothlin also interprets the symptoms as due to central action (72).

In mice, the effect is chiefly to cause exophthalmos and erection of the hairs. Death from fatal doses is preceded by clonic convulsions (20).

Action on the uterus. As pointed out by Brown and Dale, ergometrine was discovered because of the fact that the human puerperal uterus was caused to contract by doses of ergometrine having no other perceptible effect, and the action on the uterus, especially in the postpartum state, is the only specific effect produced in laboratory animals by small doses.

Human uterus. In a general way the actions of all of the physiologically active ergot alkaloids are alike. The peculiar characteristic of the new water soluble alkaloid ergonovine (ergometrine) is the ease and promptness with which effects are produced after oral administration. Both Davis and Moir have recently given descriptions of the effects, based on their clinical experiences (19, 62). The following description is taken from the paper by Moir.

> The essential effect is the production of a rapid series of contractions which, after sufficient dosage, follow each other so closely that the uterus as a whole has no time to relax, and a state of spasm therefore results. It must be noted, however, that great muscular activity continues throughout this stage. As the effect passes off, the contractions again become spaced out, and again the uterus may be able to relax between individual contractions. This second stage tends to persist for many hours.

The onset of effects from the oral administration is in seven or eight minutes, after intramuscular injection in three or four minutes, and after intravenous administration in less than a minute. Although it seemed to be the earlier belief that the action was transient, both Moir and Davis indicate that the action may persist for a number of hours.

Guinea-pig uterus. The isolated uterus of the virgin guinea pig is peculiarly sensitive to most oxytocic substances. It is stimulated by small amounts of ergonovine, (96, 72, 20) sometimes in dilutions as great as $1:1x10^9$ (7), but there is no preferential sensitiveness as compared to ergotoxine (7). Since the usual reaction of the guinea pig uterus to sympathetic stimulants is inhibitory, this action of both ergonovine and ergotoxine is probably to be considered a direct stimulation of the muscle. In the parous but not pregnant nor puerperal uterus of the guinea pig under anesthesia ergometrine caused a small increase in tone but had little effect on rhythm. In the puerperal uterus, according to Brown and Dale, there is a distinct difference between the action of ergotoxine and that of ergometrine. While there is a good deal of variation among animals, in general ergotoxine acts after a relatively long latent period (even after intravenous administration) and the effect in increasing tone is greater than that on rhythm; ergometrine has a shorter latent period, and the effect on rhythm is greater than that on tone (7).

Rabbit uterus. All workers have noted the especial sensitiveness of the rabbit uterus to ergonovine. This represents a difference from ergotoxine and ergotamine, which alkaloids are not very effective in stimulating the rabbit uterus *in vitro*. In the assay of ergot by the method described by Broom and Clark (6) concentrations of ergotoxine or ergotamine which very much depress or completely annul the stimulant action of epinephrine, are usually without any stimulating effect on the uterine strips.

The concentrations which were found stimulant by various workers vary somewhat. Thompson found that on fresh strips, concentrations of his ergostetrine of from 1:500,000 to 1:3,000,000 were effective (96). The tracing given in the paper of Dale and Brown shows a stimulant action from 1:750,000. Davis *et al.* found 1:2,000,000 uniformly gave prolonged rhythmic contractions with increase in tone (20). The non-pregnant uterus *in situ* also shows an increase in rhythmic activity after ergometrine (7).

Cat uterus. According to Brown and Dale (7) the effect of ergometrine on the non-pregnant cat uterus, which normally shows a

high degree of tone and active rhythm, is not to be differentiated from that following ergotoxine or ergotamine. The effect on the parous uterus is however more striking. Even small doses added to the isolated preparation result in marked rhythmic activity, which may last for a number of hours. Likewise the parous uterus *in situ* shows a marked rhythm and frequently a rather marked rise in tone. On the pregnant uterus Thompson found ergonovine to act much more promptly and powerfully than did either ergotamine or ergotoxine (96).

The isolated *uterus* of the *hamster* increased in tone, then relaxed, but showed a greatly augmented rhythm after ergometrine (7). The isolated horns of the *rat uterus* respond with decreased activity, as is also the case with ergotamine (72).

The post-partum *uterus of the dog* gave a distinct response to 0.005 to 0.05 mgm. per kilo (ergotocin) given intravenously; by mouth 0.5 mgm. per kilo produced within six minutes rhythmic contractions and increase in tone (20).

Other smooth muscle. Intestine. There is a variation in response of isolated preparations of small intestine, depending on the species. The intestine of the cat shows no response to ergometrine (or ergotoxine) in concentrations as high as 1:30,000 (7). The jejunum of the rabbit is inhibited by 1:100,000, although the effect is not marked. The jejunum of the guinea pig however is definitely stimulated to an increase in tone with concentrations as low as 1:1,000,000 (7). Both the inhibitory action on the rabbit's jejunum and the tonic effect on the guinea pig's jejunum are antagonized by ergotoxine, in relatively high dilution, 1:100,000 (7, 20). Thompson has reported a similar antagonism on the rabbit uterus (96). The explanation of this phenomenon is not clear. The ergotoxine effect is readily removed by washing, being much less persistent than its corresponding antagonistic action on the epinephrine response.

The *seminal vesicle* of the guinea pig is not affected by ergometrine in concentrations of 1:3,000, so far as its reactions to epinephrine are concerned (7). Rothlin has shown that this is a very sensitive structure for demonstrating the sympathetic paralysis induced by ergotamine (70). The perfused vessels of the hind legs of the frog are constricted by concentrations of 1:50,000 to 1:150,000. Greater dilutions are ineffective (20). The action on the smooth muscle of the eye is discussed elsewhere.

Circulation. Given orally the blood pressure of humans is not affected by therapeutic doses (1). Intravenously in anesthetized cats

and dogs (chloretone anesthesia) Thompson usually obtained a pressor effect, which was relatively feeble compared with that from ergotamine or ergotoxine; occasionally a depressor effect was obtained (96). When ether was used as the anesthetic, 0.1 to 0.5 mgm. of ergotocin had little effect in cats, but larger doses (1.0 to 30 mgm.) usually gave a fall, which was not prevented by atropine. In pithed cats 0.5 to 1.0 mgm. gave a rise in pressure (20). Brown and Dale observed a similar rise in spinal cats, whereas in cats under ether or chloralose ergometrine gave a fall in arterial pressure, both marked and prolonged. Even in spinal cats, where the first injection gave a pressor effect, the second injection usually gave a fall (7). Rothlin reported observations essentially in agreement with these findings. He also observed that the rise of pressure resulting from clamping the carotids is reduced by ergotamine but not by ergonovine. Depressor stimulation is less effective after ergonovine than before, but again the action is much less than that of ergotamine (72). Raymond-Hamet studied the effect of ergometrine on the volume of the kidney in dogs under chloralose. While repetition of dosage gave less marked rises in blood pressure, the renal constriction was both marked and more prolonged than after the initial injection (65).

The cardiac action does not seem to be important. Rothlin found no action in the heart-lung preparation, even in large doses (72). Brown and Dale observed no action on the rabbit heart perfused by the Locke-Langendorff technic, in doses up to 1 mgm. (7). In the perfused frog heart however a decrease in both rate and amplitude is reported (20). The vascular action has not been extensively studied. Addition of ergometrine to the perfusion fluid passing through the hind limbs of a cat resulted in a small preliminary constriction followed by a prolonged relaxation of vascular tone (7). Perfusion of the rabbit's ear resulted in a constriction more marked than that following ergotamine (72). The action on frog vessels and renal vessels in the dog was mentioned in the preceding paragraph.

Action on the cock's comb. The typical bluing and cyanosis of the cock's comb has been shown after ergonovine by several investigators (7, 20, 72, 96). It differs however in both rate of onset and recovery from ergotoxine and ergotamine. Particularly is this true when the substance is given orally. Brown and Dale found that after oral administration the onset of action could be detected in five minutes, while the earliest effects of ergotoxine administration were present only 45 to 60 minutes after administration. These lasted in some degree

until the next day, while the effects of ergometrine began to fade from about the third hour, and had completely disappeared by the next day. These investigators failed to produce gangrene in the comb of a rooster given by injection on successive days, 15, 10, 10, 20 and 10 mgm., a total of 65 mgm. (7). Rothlin also was unsuccessful in producing gangrene (72). But Davis *et al.* gave 0.5 mgm. ergotocin maleate twice daily, by intramuscular injection, for 24 days, to 6 roosters, three of which developed gangrene of the comb (20).

The degree of discoloration produced by ergotoxine is used in the Pharmacopoeial assay of ergot in the United States. Using this method, Thompson compared his alkaloid with ergotoxine ethanesulphonate, and found that crystalline ergostetrine (base) had a potency of approximately 160 per cent (±20 per cent) of the standard ergotoxine salt (96). Davis *et al.* reported three lots of "ergotocin maleate" to be 135, 125, and 143 per cent of the activity of ergotoxine ethanesulphonate (20). Subsequent "factory lots" gave in most cases values about the same as the ergotoxine salt (93). Brown and Dale report that the onset after injection of ergometrine was a little prompter than from the other ergot alkaloids, but that the maximum effects were of about the same intensity (7). The doses which they apparently used, 0.75 to 1.0 mgm. per kilo, were quite large, and it is probable that the differences of the order reported by the American workers would be concealed by these doses. The symptoms which follow ergonovine are similar to those seen after ergotoxine or ergotamine.

Respiration. In the unanesthetized cat or rabbit, ergometrine is followed by a very marked increase in frequency. The same acceleration occurs in the anesthetized rabbit, but in the cat under ether there is a marked depression of both rate and amplitude (7).

Metabolism. In white rats 1 to 3 mgm. per kilo intravenously were without effect. Five milligrams per kilo gave increases of from 25 to 39 per cent, and 10 mgm. increases from 52 to 81 per cent (20).

Temperature. The temperature of the mouse is lowered, there is no clear cut effect in the guinea pig, in the rabbit and cat the temperature is elevated.

Kidney. Zunz and Vesselovsky found that intramuscular injection into unanesthetized dogs was diuretic. It increased the rate of elimination of urine whether water alone, salt solution, or solution of urea had been given (104, 108). Under the same circumstances ergotamine acted in the opposite direction (102). Clinically the amounts given do not seem to modify urine secretion (1).

Eye. The mydriasis and exophthalmos induced in cats and rabbits have been mentioned in a preceding paragraph. As noted there Brown and Dale believed that the mydriasis was in part due to a central action. These workers did however find that an isolated cat's iris dilated on addition of ergometrine (7) and Davis *et al.* reported dilatation with local application of ergotocin in the rabbit (20). Since under these last conditions the light reflex was preserved, the action is apparently due to a local stimulation of the dilator fibers.

Sympathetic nervous system. The symptoms induced by ergonovine suggest a stimulation of the sympathetic nervous system. The experiments of Brown and Dale mentioned above, seem to indicate that the action is largely central. However there are a number of observations, the rise in blood pressure in the anesthetized rabbit, the inhibition of the jejunum and the stimulation of the excised uterus of the rabbit, the dilatation of the pupil, on local application—these could possibly be a peripheral sympathetic stimulation. On the other hand the stimulation of the guinea-pig uterus and intestine is quite the opposite of the effect of epinephrine. The action which so characterstic of the ergotoxine-ergotamine group, that is, the depression or reversal of the epinephrine response, is, according to all observers, almost wholly lacking. The effects of epinephrine on the blood sugar, on the blood pressure, on the volume of kidney or intestine, on the excised uterus of the rabbit, are not appreciably modified. In a comparison of the action of ergometrine and ergometrinine with respect to their ability to annul the renal vasoconstrictor action of epinephrine, Raymond-Hamet found that 5.5 mgm. of ergometrine were required, as contrasted with 24.5 mgm. of ergometrinine (66). Both of these amounts are very large when compared with the amount of ergotamine necessary to produce the same effect [$\frac{1}{30}$ to $\frac{1}{60}$ mgm. per kilo, Raymond-Hamet, (64), 0.0054 to 0.11 mgm. per kilo, Wulp and Nelson, (100)].

The table from the paper of Rothlin, summarizes these and other differences between ergotamine and ergonovine (ergobasine) (69), table 3.

Pharmacology of ergometrinine. The pharmacology of this alkaloid has not been extensively studied. Raymond-Hamet found that in the dog it caused a vaso-constriction and a rise in blood pressure. Its very weak antagonism to the renal action of epinephrine has been described in the last paragraph (66). Intravenous injection of 4 mgm. per kilo in an etherized cat produced a prolonged fall in blood pressure,

TABLE 3

Pharmacological properties of ergotamine and ergobasine (after Rothlin (72))

	ERGOTAMINE	ERGOBASINE
Uterus:		
Abortion, cats, after ½–1 mgm. oral or subcut.	Positive	Positive
Action in vitro:		
Rat	Decreased activity	Decreased activity
Guinea pig	Contraction	Contraction
Rabbit	Contraction	Contraction twice as strong
Cat	Contraction questionable	Contraction questionable
Action in situ: i. v. or enteral:		
Rabbit, Cat	Increase in tone and peristalsis	Increase in tone and peristalsis
Circulation:		
Blood pressure:		
Rabbit, narcotized:		
Small doses	Weak rise	Rise, stronger
Large doses	Fall	Rise, secondary fall
Rabbit, without narcosis		Rise, stronger than in narcosis
Cats, narcosis i. v.	Rise	No rise, rather a fall
Reflex cats	Rise	Rise, but weaker
Heart-lung preparation:		
Cats		No action from 2-3 mgm.
Vessels:		
Perfused rabbit ear	Contraction	Contraction more marked
Cock's comb	Cyanosis, gangrene	Cyanosis, no gangrene
Depressor phenomenon:		
Rabbits i.v	Reduced, 3× stronger than ergobasine	Reduced
Cats		
Closing of carotids:		
Rabbits, Cats	Reduced rise	No reduction
Respiration:		
Rabbits and cats without narcosis	Increased frequency, decreased volume	Increased frequency, decreased volume
Rabbits, narcotized	Same, large doses required	Same, larger doses required
Cats, narcotized i. v.	Depression to standstill	More marked
Body temperature:		
Mice, subcut.	Fall	Fall
Guinea pigs, subcut.	No clear effect	No clear effect
Rabbits i. v.:		
Small doses	Fall	Rise
Large doses	Rise	Rise
Cats, subcut. large doses	Rise, weaker than ergobasine	Rise
Blood sugar:		
Rabbits, Dogs	Without visible effect; decrease in epinephrine hyperglycemia	Moderate hyperglycemia and increase in epinephrine hyperglycemia
Sympathetic functions:		
Rabbit uterus	Epinephrine reduction or reversal	No reduction or reversal

TABLE 3—*Concluded*

	ERGOTAMINE	ERGOBASINE
Sympathetic functions—*Cont'd:*		
Guinea pig seminal vesicles	Epinephrine reduction	No reduction or reversal
Blood pressure, cats	Epinephrine reduction or reversal	No reduction or reversal
Vessels of kidney	Epinephrine reduction or reversal	No reduction or reversal
Vessels of intestine	Epinephrine reduction or reversal	No reduction or reversal
Pupil:		
Cat	Narrowed	Widened

with bundle branch block and tachycardia (8). The stimulant action of epinephrine on the excised rabbit uterus was not antagonized by concentrations up to 1:27,777. This concentration was somewhat stimulant to the muscle; the potency was determined to be about 1 per cent of that of ergotocin maleate. On the cock's comb the potency was even less than 1 per cent. Isolated rabbit intestine was inhibited. A 1 per cent solution did not dilate the rabbit's pupil (8). The effect on diuresis in the unanesthetized dog with permanent bladder fistula was found to be similar to that of ergometrine, but very much weaker (105).

Pharmacology of ergoclavine and sensibamine. Pharmacological studies of these two substances are not numerous. Kreitmar, working with ergoclavine (55), and Vartiainen studying both alkaloids (99), reached the conclusion that they did not differ from ergotoxine or ergotamine either qualitatively or quantitatively in their actions. Rössler and Unna believed sensibamine to have the same actions, except that the effects on temperature were more like those of ergotoxine than those of ergotamine (68).

The findings that sensibamine is quantitatively like ergotamine, are difficult to reconcile with the views of Stoll, that this alkaloid is an equimolecular mixture of ergotamine and ergotaminine, since ergotaminine has been found clearly less active than its isomer (107, 67). Rothlin however found sensibamine and ergoclavine to have about one half the activity of ergotamine on the rabbit's uterus *in situ*, and on the depression of the epinephrine response of the isolated rabbit uterus, which findings are consistent with the views of Stoll (73).

Sensibamine is intermediate between ergotamine and ergotaminine in its action on diuresis in the dog (110). Ergoclavine resembles ergotamine, since it reduces markedly the amount of urine in bladder fistula

dogs, either fasting or after water or sodium chloride solution administration (103, 107).

Ergosine and *ergosinine.* Pharmacological studies of these substances have not come to the reviewers' notice. If however the view of Barger is correct, that ergoclavine is an equimolecular combination of the two alkaloids, then the pharmacological findings reported for ergoclavine would indicate that the actions of ergosine and ergosinine are similar to those of ergotamine and ergotoxine.

Ergine. Rothlin (72) and Zunz and Vesselovsky (106, 109) have examined this degradation product of the older alkaloids, pharmacologically. The latter authors observed neither salivation nor vomiting in dogs, given 0.01 mgm. to 0.12 mgm. per kilo. However the pupil dilated, there was an increase in respiratory rate, tremor and agitation. Intramuscular injection did not modify carotid blood pressure, but intravenous injection of 0.03 mgm. per kilo gave a moderate and transitory rise. Doses of 0.1 mgm. per kilo did not bring about a reversal of the hypertensive action of epinephrine in dogs under chloralose. The action on water diuresis was variable, but the chloride content of the urine tended to decrease. Rothlin found that the hyperthermic action in rabbits was equal to that of ergobasine, but that the effect on the rabbit uterus was very slight, as was the antagonism for epinephrine (73).

Ergotism. The cause of the convulsive type of ergot poisoning has not been clearly established. Barger, in his monograph, discussed in detail the possibility of a deficiency of vitamine A as a contributing factor. Experimental attack on the problem, other than that of Mellanby, mentioned by Barger, does not seem to have been made. All of the reports on experimental poisoning have to do with the gangrenous type, except for that of Stockman. This author believes that the symptoms of convulsive poisoning are due not to ergot, but to rye. He was able to produce a picture in monkeys, said to resemble convulsive ergotism in man, by feeding them on rye, and more rapidly, by oral, hypodermic, or intravenous administration of substances which he extracted from grains, said to be salts of phytic acid, or decomposition products of the same. On the other hand, two monkeys were not made to show the gangrenous type of ergot poisoning when fed for two months on cakes made from ergotized rye together with fresh fruit (86).

Gangrenous ergotism is readily produced experimentally in the tail of the white rat, and in the rooster's comb, by injection of ergotoxine or ergotamine (69). Langecker failed to produce any symptoms by

administering ergotamine tartrate to white rats, approximately 6 to 7 mgm. per kilo, given in the diet, over a period of two months. Feeding of ergotized rye was however effective in producing chronic ergotism, especially in rats (57). McGrath has made the very interesting observation that the injection of theelin protects female rats against the gangrene producing effect of ergotamine, while male rats under similar conditions were incompletely protected (59).

The pathology of experimental gangrene has been studied by a number of workers. Kaunitz (49) called attention to the similarity between thrombo-angiitis obliterans and the gangrenous type of ergot poisoning. McGrath (59) and Lewis and Gelfand (48) describe the histological changes in detail. The latter studied the changes induced in the comb of white Leghorn hens by the daily injection of 10 mgm. of ergotoxine. The resultant constriction of the arteries of the comb could not be relaxed by warming. The flow did not completely stop, but the disturbances of nutrition led in a few days to injury to the intima, with loss of plasma and stasis in the capillaries. In the central vessels clotting occurred. The pathological changes were similar to those which followed mechanical obstruction of the blood flow through the comb. It was concluded that the nutritional disturbances induced by the spasm and the decreased flow lead to secondary changes in the vessels, which in turn are responsible for arrest of the circulation, and death of the tissues.

Clinically, gangrene of varying degrees has been reported, most frequently in cases of puerperal sepsis treated with ergotamine. Since gangrene may also occur in the absence of drug treatment, not all observers have felt that ergotamine should be implicated. Moir says of the reports: "Perusal shows the question is not settled, but one is left with an uneasy feeling that a strong suspicion is attached to administration of ergotamine or ergotoxine as one of the chief causes of production of puerperal gangrene" (61). The clinical literature has recently been reviewed by Yater and Cahill (101), who reported bilateral gangrene in a male subject receiving ergotamine tartrate for pruritis. Over a period of $6\frac{1}{3}$ days a total of 0.0095 gram was given, 0.5 mgm. at a dose, hypodermically. On the 13th day after the beginning of the administration, the toes of both feet developed gangrene, with a definite line of demarcation. It seems fairly clear that in this patient only the ergot alkaloid can be blamed for the action.

The assay of ergot. The assay of ergot is a very complicated matter, and a detailed discussion is beyond the province of this review. The

presence of both groups of active alkaloids, as well as their inactive isomers, makes the use of physical or chemical methods, which do not distinguish between the active and inactive members, theoretically unsound. However, according to Barger, a rough estimate of the activity can be obtained by a determination of the total alkaloidal content. A second source of difficulty lies in the apparently wide variation in the content of water-soluble alkaloid. Hampshire and Page (27) found up to 20 per cent of water soluble alkaloid in Spanish ergot. On the other hand Stoll obtained *no* ergobasine in Russian and Polish ergot, finding it only in Spanish, and to a lesser extent in Portugese ergot. According to Stoll the yield of ergobasine did not exceed 60 mgm. per kilo, whereas from a good ergot, as much as two grams of ergotamine could be isolated (87).

The chemical methods which have been used are 1, determination of total alkaloids by extraction methods, either weighing or titrating; 2, determination by colorimetric methods based on the blue color given with p-dimethylamino-benzaldehyde, the color intensity being the same for equimolecular amounts of the eight alkaloids; 3, determination by the ultraviolet absorption spectrum. These methods all determine total alkaloids. Hampshire and Page (27) have attempted to determine the relative amounts of water soluble and water insoluble alkaloids, by these differences in solubility, estimating the amount of each fraction by the color reaction. The amount of ergometrine by this method in Spanish ergot in a series of samples was: 24, 16, 20, 20, and 19 per cent; in two samples of Russian ergot, 13 and 21 per cent. Rothlin questions whether this large percentage of water soluble alkaloids can be due only to ergometrine (74).

Of the biological methods, two have been most extensively used, the cock's comb method, official in the United States Pharmacopoeia XI, and the so-called "Broom-Clark" method, based on the ability of the ergotoxine-ergotamine group to antagonize the stimulant effect of epinephrine on the excised rabbit uterus. The second method is quite accurate in its determination of the sympathicolytic action, but since this action is practically lacking in the water soluble group, the method is not applicable. Swanson, Hargreaves, and Chen (93) used a variety of methods, cock's comb, isolated rabbit uterus, postpartum dog uterus, postpartum human uterus, colorimetric and polarimetric, for evaluation of the water soluble fraction (ergotocin) after its separation, and found the isolated rabbit uterus, supplemented by the clinical method, satisfactory. Since the stimulant action of ergonovine on the rabbit uterus

is antagonized by ergotoxine, the method could not be used to determine total oxytocic activity of a galenical ergot preparation. No statements regarding the possible antagonism of ergotoxine or ergotamine for ergonovine in the clinical use of ergot have come to the reviewers' attention.

Rothlin found that the rabbit uterus *in situ* was a satisfactory organ for revealing differences between the different alkaloids (74). An assay method using this structure does not seem to have been described.

It seems possible that a combination of chemical methods of separation, with biological methods of determination of the activity of the separated fractions, might serve as a way of evaluating galenical ergot preparations as to their clinical potency. However, a substitution of the pure alkaloids would do away with the necessity for any such determination.

It is not possible in the space of this review to mention the large number of papers in which ergotoxin or ergotamine have been used as tools of research, nor can the clinical uses be discussed.

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